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New Technologies for the Utilization of Biologically Based Raw Materials for Feed and Food Production

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NEW TECHNOLOGIES FOR THE UTILIZATION OF BIOLOGICALLY BASED RAW MATERIALS FOR FEED AND FOOD PRODUCTION

Proceedings of a Task Force Meeting
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FOREWORD

Within the activities undertaken by IIASA's Food and Agriculture Program one part has been directed to assessing the role which new technologies for protein production could play in the future in covering the global demand.

This report contains the main papers submitted to the Task Force Meeting on *"New Technologies for the Utilization of Biologically Based Raw Materials for Feed and Food Production"* held at Tbilisi, Georgia, USSR in August 1981. The meeting was the second in a series of meetings dealing with the problems of new technologies for the utilization of agricultural wastes.

The main topics for discussion at the Tbilisi meeting were defined during the first meeting held at IIASA in September 1980. Furthermore the network of collaborating institutions and teams, established after this first meeting, produced interesting background material in the form of answers to the questionnaires distributed by IIASA. (See Table 6 of this report).

The meeting was seen as a further step towards the assessment of the new technologies on protein production and the basis for future collaboration was outlined and the proposal for holding the next meeting was submitted.

Kirit S. Parikh
Program Leader
Food and Agriculture Program

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The editors are grateful to all those who contributed to the Task Force Meeting, whether by formal presentation, or through participation in the discussions.

Grateful acknowledgement is made to experts from the Soviet Committee for Systems Analysis and to Professor G. Mikeladze and his team at the Tbilisi State University for the excellent preparation of the meeting.

In particular, the editors would like to thank Professor Mikeladze, Dr. J.T. Worgan and Dr. R. Oganov for their help and valuable comments in putting this volume together.

Special thanks are due to Julia Czekierska for her help in organizing the meeting, and the preliminary editing as well as typing the final manuscript with the help of Cynthia Enzlberger.

CONTENTS (The names of authors who participated in the Task Force Meeting are underlined)

* *These papers were distributed at the meeting although their authors were not able to participate*

THE ANALYSIS OF NEW TECHNOLOGIES FOR THE UTILIZATION OF AGRICULTURAL AND FOOD PROCESSING BY-PRODUCTS AND WASTE MATERIALS AS A PART OF IIASA'S FOOD AND AGRICULTURE PROGRAM.

S. Münch and J. Hirs

The Problem

One of the most acute problems in the world today is that of human nutrition. Considerable progress must be made in the field of food production to irradicate hunger and malnutrition among the underprivileged classes in the developing countries and to establish a nutritional basis which will be able to sustain a world population anticipated to reach 6 - 6.5 milliards by the turn of the century. It is not only vital to satisfy the demands for energy-giving foods but also nutritional requirements, particularly adequate supplies of protein. Protein is an essential component of the diet, without which human beings suffer both in physical and mental health.

Efforts to produce more food from traditional sources have been made in many directions: plant genetics, animal husbandry, development of marine and inland water fisheries, etc. Undoubtedly, intensification of agriculture will remain the basis for improving food supplies in the foreseeable future. At the same time, demographic growth on the one hand, limited land and fresh water resources, rising costs for energy and other intensification factors on the other, will require increased efforts to make additional sources accessible for direct human consumption or a growing number of productive livestock.

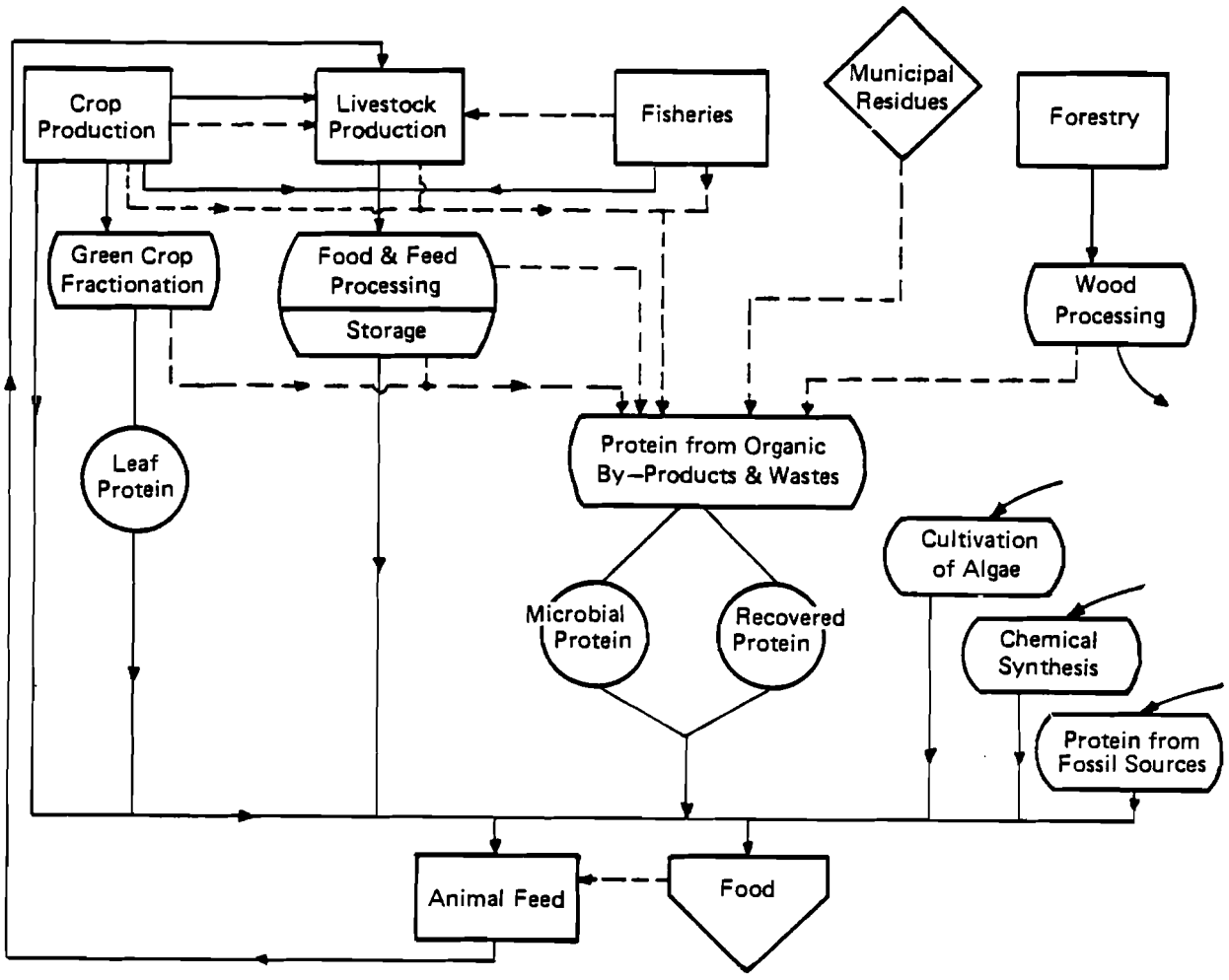
Assuming that in the coming decades the demand for animal products and consequently also animal feeds will increase, the discovery of alternative protein sources could considerably or even entirely reduce the use of wheat, soya, etc. as fodder and thereby make valuable primary products from plants directly available for human consumption.

That is why it is not only necessary to improve agriculture and food production technologies currently being applied, but also to look for new, unconventional methods which are suitable for the production of protein and nutrients and which will be adequate to meet the growing global and regional demands.

The main directions in the probable development of animal feeds and human food from traditional and novel sources are indicated in Figure 1.

Figure 1:

PRODUCTION OF EDIBLE PROTEINS FROM AGRICULTURAL AND OTHER SOURCES



As far as the availability of resources in a given region are concerned, different substances have been proposed for increasing protein supply: petroleum and natural gas, green crops, agricultural and food processing residues, wastes from the paper industries, municipal residues, etc. (Table 1). In order to use these and other substances as raw materials for obtaining proteins, a number of different technologies are feasible or are even being applied on different production scales, for example:

-- production of single cell protein (SCP) from fossil fuel sources
-- conversion of biomass to microbial protein
-- biological synthesis of protein (algea)
-- extraction of proteins from crop plants (LPC)
-- recovery of protein from agricultural, industrial, or municipal wastes
-- improving the nutritional value of protein from plant sources
-- use of cellulosic substrates for growing mushrooms

Although the chemical synthesis of nutrients for food or feed is also scientifically feasible, the amount of energy consumed by presently known processes still requires further investigation for the elaboration of economically efficient technologies for large scale production. On the other hand, production of SCP from petroleum and natural gas is under way in many countries (USSR, Japan, England, etc.). Large scale synthesis of SCP on a hydrocarbon base has already reached a high technological niveau.

In this respect the use of natural gas has proved to be particularly efficient. But there are, however, competing demands for the fossil fuels and it is well known that supplies are not unlimited. Therefore, research into other prospective raw materials for the production of microbial protein has been intensified over the last years.

One of the most promising ways of reducing protein deficiency is by means of microbial conversion of by-products or wastes from agriculture, forestry, agro-industries and fisheries to protein concentrates. Bio-resources of this type exist in every country and are constantly being renewed in plants by photosynthesis. As an indication of the quantity of these secondary products, it is estimated that agricultural residues constitute approximately two-thirds of total crop production. Estimated quantities of some agricultural, forestry and food or feed processing residues are given in Table 2.

Some secondary products are recycled into the food production system as livestock feed and only a very small quantity are directly incorporated into food products. A considerable proportion, however, of these secondary products is unsuitable as livestock feed, or has a low nutritional value. Furthermore, some waste materials, particularly those which occur as factory effluents, create a pollution problem. Apart from these, there are some other advantages which emphasize the increasing significance of microbial conversion processes as alternative technologies for protein production:

Table 1. Conventional and Novel Sources of Nutrients with a High Protein Content.

Source	Type	Examples
	Oilseeds	Soyabeans, peanuts, rapeseed, cotton seed, sesame seed, sunflower seed
Agricultural or equivalent	Legumes (other than oilseed)	Broad bean (vicia faba)
	Leaf protein	Various types of leaves
	Protein from farm animals	Meat, milk eggs
Fishery and Aquaculture	Protein from aquatic animals	Various types of fish and crustacean
	Algae	Spirulina, Chlorella and other types of algae
Biosynthesis	Carbohydrates (residues from agriculture, food, industry, and wood processing, municipal wastes, etc.)	Microproteins (bacteria or fungi)

Table 2. Annual World Production of some Agricultural and Processing Residues.

Type of Residue	Quantity of carbohydrate (x 10^3 ton)
Wheat straw	286.580
Wheat bran	57.320
Maize stover	120.040
Maize cobs	30.070
Sugar cane bagasse	83.000
Molasses	9.300

Source: J.T. Worgan. In: Proteins in Human Nutrition (J.W.G. Porter and B.A. Rolls, eds) London 1973.

-- The protein content of the products of microbial conversion is significantly higher than that of traditional food or feed (Table 3)

-- The extremely high rate of microbial synthesis enables some micro-organisms to reproduce in a very short time. Microbes can double their cell mass in 20 minutes to 6 hours. Yeast can double their cell mass in about 2 hours.

-- Production is exceptionally independent of climate, weather conditions and the ravages of pests and diseases which may reduce or completely destroy yields of agricultural crops.

-- Microbial conversion processes do not compete with agriculture for arable land.

Increasing attention is being given to the addition of algae in human nutrition or as protein animal feed in livestock production. There are more than 100.000 species of these plants, and most of them have yet to be explored for possible uses. Already a few of them are eaten in various parts of the world or processed to high protein food commodities sold in health food stores. Favorable natural conditions in tropical and subtropical regions open up new prospects for cultivating some types of algae which can be used as substitutes for conventional nitrogen fertilizer.

Because micro-organisms are capable of a wide variety of metabolic reactions they can adapt to many sources of nutrients. This adaptability makes them suitable not only for industrial but also for small scale fermentations. The last method mentioned already applied thousands of years ago for processing and preserving foods, beverages or animal feed is still important for improving the food supply in tropical and subtropical regions. At the same time industrial biosynthesis is developing feasible technological alternatives to bridge the energy/protein gap in food deficient countries as well as for reducing the dependence of some developed countries with a highly intensive livestock sector on imports of cereals as well as of soyabeans and other protein concentrates for animal feed.

Whereas highly developed industrial technologies are known for and used for obtaining protein concentrates from fossil fuel sources, most of the technologies considered for microbial conversion of residues from agriculture, processing industries or equivalent sources are still at the laboratory stage or applied at a pilot plant level (Table 4). A number of varied and complex scientific, technical, economic, medical and even social problems must be solved to make the broader use of the promising properties of microbial conversion of biomass feasible as an economically significant alternative to traditional technologies.

Table 3. Average Protein Contents of Raw Foods

Type	%	Type	%
Cassava	1-2	Beef	18.0
Potatoes	2.1	Chicken	19.0
Milk (liquid)	3.3	Roasted Peanuts	28.0
Wheat flour	11.0	Skimmed milk powder	36.0
Eggs	12.0	Soyflour	50.0
White fish	16-20	SCP	< 50.0

Sources: D. Crabbe, S. Lawson. The World Food Book, London & New York, 1981.
P. Davids (ed.) Single Cell Protein, London 1974.

Table 4. Status of Bioprotein Technologies*

Technology \ Raw material source	urban sewage	municipal wastes	animal wastes	crop residues	wood residues	processing residues	crops	livestock products	fishery products	algae	fossil fuels
Cultivation/ Reproduction							3	3	1-3	1-2	
Fractionation of Protein from Plant Biomass				1			3			0	
Protein Recovery	0-1	0-1	1-2	1-2		3	1-2	2	3		
Microproteins from carbohydrate sources		1-2		1-2	2	2	1			0	
Microproteins from hydrocarbons											2

0 - Latent: little known work but process believed to be possible
1 - Under research (laboratory stage, pilot plant)
2 - Applied in a few units of production or on a regional scale
3 - Extensively produced or applied

* The figures are based on replies to the questionnaires sent out by IIASA (Table 6) as well as estimates by the authors themselves. Values given indicate relative status rather than precise, absolute status.

The above mentioned brief description of the complexities involved clearly shows that a systems analytical approach might help towards the solution of this comprehensive problem. One way of finding an optimal approach is by means of a broad based analysis of global, national and regional achievements in protein production by traditional and non-traditional technologies respectively. Such alternatives have to be taken into consideration as the use of biomass for energy production or as fertilizer in order to guarantee effective use of all resources in the framework of an indefinite sustainable system.

Activities of IIASA's Food and Agriculture Program

As a start to research work on the Task "Technological Transformation in Agriculture--Resource Limitations and Environmental Consequences", it was suggested that a special Task Force Meeting be organized.

The focus of IIASA's Food and Agriculture Program has been on obtaining and analyzing national and international policy options to alleviate present food problems and to prevent future ones. In doing this the Program addresses both the short and long-term problems related to the development of agricultural production.

The investigation of the long-term aspects of the food problem has focused on identifying alternative paths of technological transformations of agriculture, in the light of limited resources and environmental consequences, that can lead to a sustainable, resilient and equitable world able to feed its growing population.

In this context non-conventional technologies for protein production presently being developed are being examined as an alternative source of nutrition. Although at present protein being produced by these methods is not very high, specialists are optimistic about future application. However the economic, ecological, environmental and technical aspects of the trade-off between traditional and non-traditional technologies has to be analysed. This analysis is quite complicated due to the diversity of processes and technologies under development in this field.

The Program's contribution to this analysis took the form of contacting various institutions and research groups involved in this field, collecting data on present and proposed research, and analysing the trade-off between traditional and non-traditional sources of protein production. Although only limited resources at IIASA could be devoted to these investigations, some useful activities have been initiated which resulted in an informal network being set up between IIASA and the various institutions and research groups involved in this work.

As a starting point a Task Force Meeting was organized to review present knowledge of the development and availability of non-traditional protein production technologies. This meeting was held at IIASA in September 1980 as part of the Food and Agriculture Program's activities and was entitled "New Technologies for the Utilization of Agricultural By-Products and Waste Materials".

The Task Force Meeting demonstrated the importance of the problem and the necessity of further research involving scientists from different countries and disciplines using a systems analysis approach. The presentations were of great interest from the point of view of the new technologies described, the character and nature of both the raw materials and the end products, the economic aspects, etc.*

In order to obtain the type of data required for further IIASA activities, a provisional questionnaire was prepared for the Task Force Meeting and was completed by those attending in respect of a specific example of a new technology concerned with the production of food, feed or a biological source of energy. Of the eleven replies to the key question, namely, whether it was feasible that the technology could be applied within the next 20 years, 10 positive answers were given. On the other hand, only one suggested that the technology may have negative environmental effects.

Although the answers to the questionnaire gave useful information on the technologies listed, it was suggested during discussions that more data could be obtained from an improved version of the questionnaire. It was agreed that this improved version should be prepared and forewarded not only to participants of the meeting, but also to other experts who would be interested in cooperating with the IIASA study and who would also be able to complete a questionnaire in respect of their knowledge of a particular technology. The main questions asked in the questionnaire are listed in Table 5.

The results achieved so far from the questionnaire organized by FAP's Task 2 indicates a great interest both in the industrially developed countries as well as the developing countries for an investigation of the problems connected with the introduction of nonconventional technologies for the utilization of agricultural by-products and waste materials. By the end of August 1981, 41 completed questionnaires had been received, 31 of which concentrated on processes for protein production by

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* The proceedings of the meeting have been published as an IIASA Collaborative Paper (CP-81-18).

Table 5. Main Questions asked in the questionnaire on:
"Non-conventional Technologies of Food, Feed or Bioenergy Production"

1. Designation of the Technology
   - o General description
   - o Unit operation involved in the process

2. Status of the Technology
   - o Is the process in actual production?
   - o Could a feasible production unit be established from present knowledge without further development if the capital were available?
   - o From the pilot plant studies on analogy with already established processes can an assessment be made that practice production units could be established within 20 years?
   - o Is the technology at a stage where studies on a laboratory scale suggest that a process will be feasible in the future?
   - o Does the technology still require to be tested in practice?
   - o What is the required scale of operational units? a) small; b) intermediate; c) large; d) flexible?

3. Inputs
   - o What kind of raw material (by-products, wastes) is required and how is it produced?
   - o Physical state of the raw material
   - o Main components of the raw material
   - o Are there other uses for the described raw materials?
   - o Is the raw material a significant pollution factor at one location?
   - o Is the raw material available in large quantities (global, regional)?
   - o Is the raw material produced continuously or in seasonal quantities?
   - o Can the raw material be preserved without processing?
   - o Required amount of raw material to produce one unit of product.
   - o Are there other raw materials which could be used for the technology?
   - o Labor, energy, material and capital inputs to produce one unit of product.

4. Outputs
   - o What is the use of one main product?
   - o Characteristic and qualitative composition of the main product
   - o Does the process produce other by-products?
   - o Has an adequate program of testing been carried out to establish that the product would be safe to use for: a) human consumption; b) animal feed; c) energy?
   - o If the product is a food:
     a) is it suitable for direct use?
     b) will it require further processing?

5. Environmental effects
   - o Are there positive environmental effects?
   - o What are the possible negative impacts?

6. Flow diagram of the process

Table 6. Summarized replies to the questionnaire on "Non-Conventional Technologies of Food, Feed or Bio-Energy Production".

Description of the technology	Raw Material: Kinds of raw material	Availability: global	regional	continuously	seasonal	Pollution factor: Yes	No	Status of Technology: laboratory stage	pilot plant	a few units	applied extensively	Required Scale of Operational Units: small	intermediate	large	flexible	Final products: Main product	Use of the main product: feed	food a*	food b**	energy source	Input/output ratio (in physical terms)	By-products	Observations	References
Processing of pasture/ lucerne to yield a protein concentrate and extracted herbage	Lucerne or pasture herbage	x	-	-	x	-	x	-	-	x	-	-	x	x	x	LPC (60% protein, 20% fat, 10% carbohydrate pigments)	x	-	x	-	8 mt dry or 25 mt wet material for 1 mt LPC	Protein extracted herbage as ruminant feed	In New Zealand this technology not yet applied on industrial scale	P.E.Donnelly, New Zealand
Green crop fractionation	Green forage crop	x	-	-	x	-	x	-	-	x	-	-	-	x	x	LPC	x	-	x	-	-	Protein extracted herbage	-	S.E.Heath, U.K.
Extraction of protein from leaves (PX Process)	Freshly chopped alfalfa	x	-	-	x	-	x	-	-	x	-	-	-	x	-	LPC (46% protein, 9% fat, 10% mineral substances, carotene, xanthophyll, etc.)	x	-	x	-	-	Protein extracted herbage	-	G. de Mathius, France Lucerne, France
Extraction of edible protein containing a valuable amount of carotene from leaves	Most sorts of lush green vegetation	x	-	x	x	x	-	-	-	x	-	-	-	-	x	LPC as compressed moist cake (63-65% protein, 20-30% lipid, 1-2mg beta carotene P per g) with the keeping qualities of cheese	-	x	-	-	1 mt (wet weight) of crop for 25 kg dry (100%) protein	Protein extracted herbage as ruminant feed	Raw material a pollution factor when used for silage in usual way	N.W Pirie, U.K.
Green crop fractionation	Green biomass	x	-	-	x	-	x	-	-	x	-	-	-	-	x	Protein concentrate (50-30% protein)	x	-	x	-	-	Protein extracted biomaterial		N.I.Proidak, U.S.S.R
Mechanical extraction of leaf juice to yield a protein	Green succulent herbage	-	x	x	-	-	x	-	x	-	-	-	-	-	x	LPC containing protein, fat, carbohydrate, vitamin A, xantophyll etc.	x	-	x	-	0.45 mt for 1 mt	Protein extracted herbage	Technology is similar to the PX Process	R McKenzie, Australia
Production of microbial protein (bacteria) based on methanol feedstock (KISR-SCP Pilot Plant)	Methanol by catalytic oxidation of flare gas from oil wells (methane)	-	x	x	-	-	x	-	x	-	-	-	-	x	-	Protein concentrate (up to 70% in dry weight)	x	-	x	-	0.8 mt for 1 mt f microbial biomass	-	Similar to ICI technology	I.Y.Handan, A.Prckop, H.Ratcliffe, Kuwait
Fermentation using a filamentous fungus to produce protein (Pekdo Process)	Sulphite waste liquor from the manufacture of cellulose pulp from wood, etc.	x	-	x	-	x	-	-	-	x	-	-	x	x	x	Protein concentrate (+/- 60% protein) for animal feed	x	-	x	-	+/- 8 mt carbohydrates for 1 mt protein concentrate	-	Continuous process	M Lehtomaki, Tampella Ltd Finland
Enzymetic degradation of cellulosic wastes and protein biosynthesis	Ligno-cellulosic waste materials	x	-	x	x	x	-	-	x	-	-	-	-	-	x	Protein concentrate	x	-	x	-	1:0,2	Lignin	-	C.Panayotov, Bulgaria
Production of fungal protein by submerged culture	Agriculture food processing wastes	x	-	x	x	x	-	-	x	-	-	-	x	x	x	Protein concentrate for animal feed	x	-	x	-	0.45 mt for 1 mt	-	In future by-products such as enzymes may be used	J.T.Worgan, U.K.

* actual food

** food for next 20 years

Table 6. Summarized replies to the questionnaire on "Non-Conventional Technologies of Food, Feed or Bio-Energy Production". (cont.)

Description of the technology	Kinds of raw material	Raw Material: Availability: global	regional	continuously	seasonal	Pollution factor: Yes	No	Status of Technology: laboratory stage	pilot plant	a few units	applied extensively	Required Scale of Operational Units: small	intermediate	large	flexible	Final products: Main product	Use of the main product: feed	food a*	food b**	energy source	Input/output ratio (in physical terms)	By-products	Observations	References
Production of energetic (or) protein compounds by total hydrolysis	All kinds of ligno-cellulosic materials (sawdust, shavings, straw, bagasse, etc.)	x	-	x	x	x	-	-	x	-	-	-	-	-	x	Wood sugars in form of treacle (60% sugar) for energy or protein processing (45-55% crude protein)	x	-	x	x	-	Lignin	Continuous process	J.Holota, C.S.S.R.
Production of glucose from cellulosic wastes (hydrolysis)	Cellulosic waste materials	x	-	-	x	-	x	-	x	-	-	x	-	-	-	Glucose for food and feed processing	x	x	-	-	-	-	-	G.I.Kwaltadze, U.S.S.R.
Hydrolysis of waste cellulose materials	Municipal waste mill effluents, sawdust and sludges, agricultural wastes	x	-	x	x	x	-	-	x	-	-	x	x	x	x	Glucose or other monosaccharides to be converted into SCP, ethanol, etc.	x	-	x	x	-	-	-	T.K.Nikolov, Bulgaria
Conversion of potato processing wastes into energy	Losses from potato processing and processing wastes from other food commodities	-	x	x	-	x	-	-	x	-	-	-	-	-	x	Liquid fuel	-	-	-	x	-	By-product may be animal feed	-	D.R.Heldman, U.S.A.
Saccharification and fermentation of ligno-cellulosics	Wood chips	x	-	x	-	-	x	-	x	-	-	-	x	-	x	Gasoline fuel substitute	-	-	-	x	40 dry pounds for 1 U.S. gallon	Solid lignin, liquid pentoses polymers	-	J.J.Zerbe, U.S.A.
Production of liquid fuels from the cellulosic residue from leaf protein extraction of herbage crops	Pulped and pressed pasture herbage	-	x	-	x	-	x	x	-	-	-	-	x	-	-	Ethanol	-	-	-	x	7-8 mt for 1 mt ethanol	Animal feed, chemical grade lignin, etc.	-	S.R.Vaughan, New Zealand
Farm and community scale ethanol production system	Agricultural and community wastes	x	-	x	x	x	-	-	-	x	-	x	x	-	-	Ethanol	-	-	-	x	-	Animal feed	-	J.Bartholic, U.S.A.
Gasification of wood and agricultural wastes	Small wood chips, small pieces of cellulosic agricult. wastes	x	-	x	x	x	-	-	-	-	x	x	x	x	x	Low biogas (20-30% carbon monoxide, 10-15% hydrogen gas, < 5% water vapour, 45-50% nitrogen)	-	-	-	x	-	-	-	J.Downey, U.S.A.
Biogas production by fermentation in an aerobic digester	Cellulosic waste, manure, human waste, etc.	x	-	x	x	x	-	-	-	-	x	x	x	x	x	Biogas (methane)	-	-	-	x	10 kg waste materials for 1 kg gas	Sludge as fertilizer	Decreasing raw material inputs in larger biogas plants	J.Parikh, India/IIASA
Anaerobic digestion of swine manure for recovery of methane	Swine manure or other animal waste	-	x	x	x	-	x	-	x	-	-	x	-	-	-	Biogas (methane)	-	-	-	x	-	Fertilizer	-	D.Sievers, U.S.A.
Utilization of crop residues as a fuel source in generating electricity	Straw and other cellulosic crop residues	x	-	-	x	-	-	-	-	x	-	-	-	x	x	Electrical energy	-	-	-	x	-	Ash as fertiliser	-	R.O.Heady, U.S.A.
Conversion of agro-industrial wastes into useable feed or fertilizer	Food wastes, paper, carton and plastic	x	-	x	-	x	-	-	-	x	-	-	-	-	x	Solid soil-like humus material	-	-	-	-	1.8-2.5 mt waste material for 1 mt fertilizer	Energy	-	R.M.Nateur, Kuwait

* actual food

** food for next 20 years

Table 6. Summarized replies to the questionaire on "Non-Conventional
(cont.) Technologies of Food, Feed or Bio-Energy Production".

Description of the technology	Raw Material: Kinds of raw material	Availability: global	regional	continuously	seasonal	Pollution factor: Yes	No	Status of Technology: laboratory stage	pilot plant	a few units	applied extensively	Required Scale of Operational Units: small	intermediate	large	flexible	Final products: Main product	Use of the main product: feed	food a*	food b**	energy source	Input/output ratio (in physical terms)	By-products	Observations	References
Recovery of proteins from waste water with membrane filtration	Waste water from the starch industry (potato, corn)	x	-	x	x	x	-	-	-	-	x	-	x	-	x	Concentrated protein rich solution (+/- 70% protein, +/- 10% ash, +/- 4% lipid)	x	-	x	-	+/- 50 cubic meters for 1 mt liquid feed	-	-	B.V Wenlin, Netherlands
Conversion of poultry waste into a high protein feedstuff (aerobic non sterile fermentation)	Poultry waste, molasses or other carbohydrate sources	x	-	x	-	x	-	-	x	-	-	-	x	-	x	Protein concentrate (+/- 40% crude protein)	x	-	-	-	0.9-1.2 kg poultry waste or 0.9 kg molasses for 1 kg feed	Fertilizer (25% of the original manure)	For farms with > 50,000 layers	M I. Shuler, U.S.A.
Production of feed yeast from nutrient-supplemented coconut waste water	Waste water (coconut liquid endosperma) from manufacture of dessicated coconut meat	-	x	x	-	x	-	-	x	-	-	-	x	-	x	Protein concentrate (+/- 40% crude protein)	x	-	x	-	100 l for 1 kg feed	-	-	E.J.del Rosario, Philippines
Production of mushrooms using ligno-cellulosic wastes	Straw, bagasse, cotton waste, etc.	-	x	x	x	-	x	-	-	-	x	-	-	-	x	Mushrooms (2-5% protein on a fresh weight basis) 30-47% protein on a dry weight basis	-	x	-	-	1 kg sterilized straw as substrate for 1.25 kg fresh mushrooms	Animal feed or soil mulch	Technology applied under tropical temperatures & humidities	K.Steinkraus, U.S.A.
Small-scale integrated food preservation operations - emphasizing efficient, minimum waste, resource sparing, low energy food processing and utilization techniques	Food of any type	x	-	-	x	x	-	-	-	-	x	x	x	-	x	Wholesome preserved foods	-	x	-	-	-	Biomass with industrial and agricultural potential	Utensils and supplies can be indigenous	R P.Bates, U S.A.
Process to dehydrate foods quickly in conjunction with a continuous explosion-puffing system	Fresh fruits and vegetables	x	-	-	x	-	x	-	-	x	-	-	-	-	x	Dry fast rehydratable food pieces (carrots, potatoes, apples, etc.)	-	x	-	-	Depends on moisture within product	Peels for animal feed	This process saves 40% to 60% of normal processing for hot air dehydration	J.C.Craig,Jr. USA
Integrated ethanol-dairy-methane facility with ethanol plant as power source	Coal, grain substrate for ethanol production wet grain residue for feed, livestock wastes as substrate for methane production and biogas used as a source of energy	x	-	x	-	x	-	-	-	x	-	-	-	-	x	Milk, wet residue feed, methane	x	x	-	x	-	carbon dioxide	There are a few integrated ethanol-cattle-methane units in operation. Proven technology is available on each of specific parts of the integrated system	F.E Hatfield, U.S.A
Combined alcohol and feed production (renewable energy program)	Giant grass by cultivation	-	x	-	x	-	x	x	-	-	-	-	-	-	-	Liquid fuel, leaf protein	x	-	x	x	1 mt for 130 l alcohol and 70 kg crude protein	Brown extracted juice unable as fermentation media	Although both fibro hydrolysis and leaf protein plants exist further development is necessary to combine the processes	J.C Chaver, Brasil

* actual food

** food for next 20 years

Table 6. Summarized replies to the questionnaire on "Non-Conventional
(cont.) Technologies of Food, Feed or Bio-Energy Production".

Description of the technology	Raw Material: Kinds of raw material	Availability: global	regional	continuously	seasonal	Pollution factor: Yes	No	Status of Technology: laboratory stage	pilot plant	a few units	applied extensively	Required Scale of Operational Units: small	intermediate	large	flexible	Final products: Main product	Use of the main product: feed	food a*	food b**	energy source	Input/output ratio (in physical terms)	By-products	Observations	References
Microbial protein production from industrial waste	Organic wastes from food and drink processing	-	x	x	-	x	x	x	-	-	-	-	x	-	x	Animal feed containing SCP, carbohydrates, lipids, nucleic acids, vitamins, etc.	x	-	-	-	60 mt liquid waste for 1 mt dry weight SCP	-	Pilot plant to be installed (1981), continuous process	R.Marchent, U.K.
Microbial feed processing using cellulose as substrate	Cellulose waste materials	-	x	x	x	x	-	-	x	-	-	-	-	x	-	Protein rich animal feed	x	-	-	-	-	-	-	K.Steinkraus, U.S.A.
Fermentation of food processing by-products	Oilseed press cake and other food processing by products	x	-	x	-	x	-	-	-	-	x	x	x	-	x	Protein rich food or feed	x	x	-	-	-	-	-	K.Steinkraus, U.S.A.
Nutritional protein improvement of high starch substrates by fermentation	Tapioca waste, cassava, manioc, yuca, etc	-	x	x	-	x	-	-	-	-	-	x	-	-	-	Protein enriched starchy substrates	x	x	-	-	-	-	Extensively applied in Indonesia	K.Steinkraus, U.S.A.
Fermentation of cereals and legumes to improve nutritive value	Any cereal or legume	x	-	-	x	-	x	-	-	x	-	x	x	-	x	Protein enriched nutrients	x	x	-	-	1:1	-	-	M.Fields, U.S.A.
Biological processing of whey (lactate fermentation with addition of ammonia water)	Whey from cheese production	x	-	x	-	x	-	-	-	-	x	-	x	x	x	Protein rich animal feed in liquid form (ammonium lactate, whey proteins, and biologically active substances)	x	-	-	-	20 mt whey for 20.5 mt liquid bioproduct	-	-	N.Kirov, Bulgaria
Isolation of biologically active compounds from wine yeast precipitates	Wine yeast precipitates	-	x	-	x	-	x	-	x	-	-	x	-	-	-	Protein concentrate (protein, carbohydrates, lipids, etc.)	x	x	-	-	-	-	-	L.A.Mudjiri, U.S.S.R.
Recovery of protein from food process effluents	Effluents from meat, poultry and fish processing plants containing proteins and fats	x	-	x	-	x	-	-	x	-	-	-	x	x	-	Protein concentrate for animal feed (60-80% protein, 10-30% fat, 5-20% ash)	x	-	x	-	1 cubic m. ter effluent for 2-4 kg concentrate	-	-	R.A Grant, U.K.
Recovery of biomass from waste streams of food chain	Losses from potato and apple processing, etc.	-	x	x	-	x	-	x	-	-	-	-	-	-	-	Animal feed	x	-	x	-	-	-	-	D.R.Heldman, U.S.A.
Low cost extrusion technology	Rice bran from milling of white rice	x	-	x	-	-	x	-	-	x	-	x	x	-	x	20% oil, (for human consumption), 18% protein	x	x	-	-	-	-	-	R.M Saunders, U.S.A.
Combined sewage purification and SCP production	Waste water from livestock production units	x	-	x	-	x	-	x	-	-	-	-	-	x	-	Purified waste water and SCP	x	-	-	-	-	-	Biogas production is also possible, continuous process	M.Ringpfeil, G.D.R.

* actual food

** food for next 20 years

microbial conversion or mechanical extraction of biomass, as well as on technologies for the improvement of the nutritive value of food and feeds. The rest of the questionnaires describe technologies for protein processing from fossil fuel sources, for generating electricity, etc. The answers received are divided regionally as follows:

Australia	1	India	1
Brazil	1	Kuwait	2
Bulgaria	3	N.Zealand	2
CSSR	1	Netherlands	1
Finland	1	Philippines	1
France	1	U.K.	5
GDR	1	USA	17
		USSR	3

A preliminary evaluation of the completed questionnaires has been made in Table 6. It illustrates what a wide range of resources can be made accessible for direct or indirect human nutrition or production of energy through a systematic use of agricultural, forestry and industrial by-products and residues as well as municipal wastes and sewage.

The most important conclusions to be drawn from Table 6 are:

-- Although it may be feasible to produce quantities of micro-proteins in the immediate future marketable for human consumption, the most extensive use will probably be the production of protein concentrates for livestock feed.

-- In nearly all countries of the world sufficient raw materials are available for the introduction or expansion of non-conventional processes of protein extraction.

-- Decisive for the speed of implementation of these new technologies will be:
  - o the assimilability, palatability and social acceptance of the final product for human consumption or animal feed
  - o the continuous availability of raw materials for large scale production
  - o the development or introduction of technologies which facilitate the production of standardized microbial protein which can be produced cheaper than comparable traditional products
  - o the development of technically and economically acceptable modes of transport for large quantities of bulky raw material (straw, sewage wastes, etc.)

-- Some of the technologies mentioned in Table 6 for obtaining microbial protein or improving the nutritional values of foods for human consumption or animal feeds are especially well suited for small scale operational units with a flexible operational scale.

Their further development could effectively support the endeavours of the food deficit countries to improve their energy/protein balance.

From an economical point of view, a comparative analysis of conventional and novel microbial technologies has to be considered and the advantages resulting from the application of non-conventional technologies evaluated in respect of saving agricultural land and positive implications for the environment (reduction of pollution, etc.).

The Second Task Force Meeting

In accordance with the recommendations of the first Task Force Meeting, a second meeting was held in Tbilisi from 25th to 27th August, 1981. This meeting was jointly organized by the Committee for Systems Analysis of the Presidium of the Academy of Sciences of the USSR, the Tbilisi State University and IIASA's Food and Agriculture Program.

The aims of the meeting in Tbilisi were to present research being currently carried out by scientists and representatives of different countries. The main purpose of the meeting was to further specify the systems analytical approach, to support coordination of research efforts of all participants, to present results of various studies, and to identify future objectives.

The contributions presented covered the following topics:

-- the microbial synthesis of protein from various wastes including effluents from industrial processes, from green crop fractionation and from the rearing of livestock;

-- the suitability of starch and ligno-cellulosic materials as substrates for microbial processes;

-- the separation of protein from leaf biomass, from wine fermentation residues and from wastes derived from the meat processing industries;

-- methods for improving the nutritional value of the protein from plant sources;

-- the evaluation of prospective raw materials for obtaining feed protein (the wastes of meat, milk, vegetable oil, and wine production and other branches of the food industry).

The previous results of IIASA/FAP activities as well as the principles of a methodology for the evaluation of non-conventional technologies were presented with the aim of carrying out systematic research.

The Tbilisi meeting illustrated the wide range of scientific disciplines involved in assessing alternative protein production technologies. To be able to evaluate the practical application of new processes and their efficiency in relation to one another, and to conventional methods of agricultural production, specific data comparable for all processes need to be accumulated. At the same time, there are essential preconditions for starting comparative analysis of alternative (traditional and non-traditional) protein production technologies. Above all, more detailed information is needed on the required inputs in terms of materials, energy, water, etc., the optimal scale of processing units and the respective capital costs, the level of labor intensity, skillfulness, etc.

Participants expressed their strong feeling that IIASA's activities in the field of nonconventional technologies should be continued and that they be considered with the long term problems of agricultural production taking into account potential resources, technological changes and environmental problems. For further work the following main objectives were suggested:

-- to study demand for food and feed protein on a global and regional scale;

-- to analyze resources, by-products and wastes of agriculture and food processing;

-- to evaluate presently available technologies and those expected to be available in future with reference to an interdisciplinary approach

The above points were recommended as the subject of a third Task Force Meeting to be held in 1982, which is mainly oriented to the Interdisciplinary Aspects of Non-conventional Protein Production.

Draft concept for the 1982 meeting on "New Technologies for the Utilization of Biologically Based Raw Materials (Wastes or By-Products) for Feed and Food Production".

In accordance with the recommendations of the Tbilisi meeting and taking into consideration the general concept of FAP's Research Plan, the following topics have been suggested for the proposed third Task Force Meeting to be held in Sofia in October, 1982, entitled:

"Systems Analytical Approach to the Assessment of Non-Conventional Protein Production Technologies (NCPT)"

The main problems to be covered by this meeting are:

-- future demand for food and feed protein on a global and regional scale;

-- the role of NCPT in meeting the protein demand during the next 2-3 decades;

-- problems of implementing Non-conventional Protein Production Technologies;

-- techno-economic evaluation of NCPT as technological alternatives for food and (or) feed production.

To enable all aspects of the given topics to be fully covered, presentations should be prepared according to the following scientific and practical questions:

-- To what extent can the adoption of NCPT for food and feed production serve to improve global and regional protein supply within the next 20-30 years?

-- What are the specific prospects and requirements for increased application of NCPT in:

  o food and feedstuff importing developed countries
  o developed countries exporting food and feed
  o food deficit developing countries without fossil fuel resources
  o OPEC countries?

-- What are the most efficient technological solutions (presently applied or expected within the next 20-30 years) for using wastes or by-products from agriculture or processing industries for animal feed or food for direct human consumption?

-- What are the problems of establishing new processes on an industrial scale?

-- In which fields do NCPT compete with other systems of production for raw materials and resources?

-- What are the feasible prospects of establishing integrative technical processes for improving overall efficiency and (or) reducing negative environmental effects?

-- What are the main criteria for evaluation of the final products of NCPT as human food or animal feed.

-- What are the social implications of introducing new protein foods?

-- What are the education and training requirements of the personnel to be involved in NCPTs?

Although it is obvious that a detailed analysis could be carried out on each of the abovementioned questions, the discussions at the Task Force Meeting will be carried out within an interdisciplinary framework, concentrating on the interrelations between specific questions.

While this may only be a modest contribution to solving the problem of hunger in the world and locating areas where more extensive research is needed, it is hoped that the meeting will be interesting and, as in the case of the past two Task Force Meetings, will contribute to closer collaboration between various institutes and to a wider interest in future research in this area.

NON-TRADITIONAL PRODUCTION OF FEED PROTEIN FROM CELLULOSE- AND STARCH-CONTAINING WASTES

A.A. Skladnev, and G.B. Bravova

Protein scarcity is a key to the earth's food problems in general. At present about 40% of the earth's population suffers from malnutrition according to FAO data. Between 30-40 million people die in a year alone as a result of starvation or as a result of deficient nutrition, mainly from a lack of protein. In the past few years the global food situation has worsened despite an overall increase in food production per head of the population. This stems from the fact that a large proportion of the population is in the lower income group and there exists a small privileged wealthy class; an unjust distribution of resources, regional specificity, and other facts are also responsible.

When further intensified use of traditional agricultural technologies is no longer effective and production fails to meet demands, non-traditional methods of food production become the focus of interest for researchers and economists. At present, the production of protein by means of microbiological synthesis seems the most promising method and has raised many hopes. This method of protein production does not depend on geographic, climatic or seasonal conditions, does not require large areas for production and utilizes renewable natural resources.

Microorganisms are very productive, a factor which predetermines the production of single-cell protein (SCP) on a large scale, and they are economic. The relatively short time needed for the production of SCP compared to the time needed when traditional agricultural technologies are employed is another advantageous factor.

It is probably not necessary to mention the quality of SCP. Many publications give evidence that SCP preparations can be recommended as feed additives as well as food products by virtue

of their composition, the correlation of amino acids, their content of vitamins, enzymes and other biologically active compounds. So the decision to intensify SCP production and the study of problems encountered can be justified because it will enable us to improve the global food situation significantly.

In some countries the industrial production of yeast protein has already been carried out. The production of so-called protein-vitamin concentrates is being carried out in the Soviet Union. The microbiological synthesis of protein has been carried out on hydrocarbon raw materials in different countries including the USSR. However, this irreplaceable natural raw material is losing in importance as a protein source because of the energy crisis and constant increases in petroleum prices.

The production of yeasts on acid hydrolysates of cellulose-containing substrates, especially wastes of the timber industry, is wide-spread. However, this method has its shortcomings: firstly, it requires the use of acid-proof equipment and is therefore comparatively low in economic efficiency.

Yeast production, although prevalent, has its disadvantages. First of all, yeasts cannot utilize complex natural substrates because the synthesis of extracellular enzymes is restricted. Yeast protein preparations are lacking in sulphur-containing amino acids, however, they have a relatively large amount of nucleic acids and this prevents their use in unlimited quantity. As a rule they may substitute at most 10% of vegetable and animal protein in a ration.

The above mentioned disadvantages are absent in microscopic fungi to a certain extent and their major advantage is that they can be grown on a variety of different and complex substrates. We are being forced more and more to consider the use of microscopic fungi as major changes have occured in the evaluation of natural substrates which should be used for the production of both protein and energy.

Cellulose is recognized as the most advantageous, economically profitable and most important renewable substrate for protein production. Because of the wide distribution and high content of cellulose in agricultural and industrial wastes, it is of great value as a raw material for microbiological synthesis. Of the microscopic fungi a large group that synthesizes cellulolytic enzymes has been located.

Of the cellulose-containing raw materials considered for the production of microbial protein are straw, the green parts of various vegetables, maize stubble, sunflower and cotton husks, wastes of the paper industry and the wastes of the food industry. Singularity of chemical structure, physical properties, transportability, locality, and seasonal and economic advantages are all characteristics which determine the choice of alternative processing methods of the raw materials such as the above named.

In our opinion it is best to divide the vegetable wastes of agriculture and industry into three groups: 1) those that can be easily transported and which are favorably located in one region; 2) those which can hardly be transported, and as a rule, containing large amounts of moisture, i.e. mostly wastes of the food processing industry; 3) agricultural wastes, especially straw, the transportation of which is unprofitable.

Large-scale methods of processing cellulose-containing raw materials for the microbiological industry are meeting with some success. Cotton husks, grape squeeze and bran are such raw materials being used. Untransportable wastes, generally the wastes of food production, must be processed at the food plants themselves, or not far from them. When processing straw it is best to use methods which can easily be reproduced on the agricultural farm scale.

As a result of investigations conducted in the Soviet Union, methods of enriching cotton husks, grape squeeze and corn bran using protein from microscopic fungi have already been worked out. When growing microscopic fungi by surface culture, the so-called dry fermentation method, the protein content increases from 3-4% to 18-20% on a cotton husk substrate; an increase to 25-28% from 5-6% is achieved on a grape squeeze substrate; and an increase from 12-15% to 30-35% is possible on a bran substrate. It should be noted that cultivation is carried out in a mechanized growing chamber and this determines the economic expediency of the method. At any rate, according to preliminary calculations, the protein obtained thus is 1.5-2 times cheaper than protein obtained on a substrate of n-paraffin of petroleum.

The protein thus obtained contains all the essential amino acids. It has been included in fodder additions for animals and has been shown to have a harmless effect. The new fodder products can substitute up to 15-20% of the whole protein amount in a ration. Besides, hydrolytic enzymes of high activity, especially cellulase, hemicellulase and pectinase, have been found in the fodder additions. The utilization of the enzymes named above in premix compositions has been shown to have positive results when fed to animals in the Soviet Union. The level of enzyme activity in the fodder obtained sometimes approaches the activity level of standard enzyme preparations.

At present it is recommended that methods of submerged culture be used for the production of protein from the wastes of the potato-processing and sugar industries. Biomass yields of up to 25 g/l with a protein content of up to 55% have been attained.

Methods of enriching straw with proteins using microorganisms have been developed and included in the economy of the Soviet Union. These methods are simple and can take place in any fodder shop or stock-breeding farm.

The technological process consists of a preliminary enzymatic hydrolysis of straw to glucose. At a fixed time of

correlation - enzyme/substrate - a content of up to 18-19% reduction compounds in the reaction mixture is regarded as a success. Furthermore, the growth of yeasts on this mixture enabled us to increase the protein content to 12% and even higher. This fodder is especially good for feeding animals because it has the following features: a moisture content of 60%; a content of nitrogen-free extractive compounds of 30% - including reduction compounds which make up 12% and wet protein also making up 12%. This method is profitable and makes possible the utilization of straw with a high level of efficiency as the protein obtained has a digestibility of 80%. The digestibility of the straw can be further increased after enzymatic processing.

In conclusion the following can be noted: the creation of a new branch of the microbiological industry - i.e. protein production on cellulose - posed a number of problems to decision-making scientists; the choice of approach to be taken has an effect on the economy and the scale of production. The question of high productivity equipment for surface culture cannot be answered, but it is precisely this method which is most expedient from the point of view of energy expenditure.

When working with microorganisms it is important to remember that their vulnerable point lies in their changeability and the possibility of variants being formed that have undesirable characters. Therefore it is necessary to select microorganisms with care. The production of microbial protein requires a 100% guarantee of the product's safety. This means that when developing the technological process, not only microbiologists and technologists, but also physicians and livestock experts must be consulted.

Table 1. Protein production by growing microscopic fungi on cellulose and starch-containing substrates

Substrate used	Method of growth	Protein content,%		loss of dry weight, %
		before processing	in the finished product	
cotton husks	surface culture	2-3	18-20	20-25
corn feed	surface culture	12-15	35-38	25-30
straw	submerged culture	2-3	10-12	-
nonstandard vegetables	submerged culture	3-4	20-23	-

Table 2. The determination of cellulase (C_1) activity in protein-enzyme preparations

substrate utilized	enzyme activity, unit/g	
	C_1	C_x
cotton husks	50	125
corn feed	28	75
rushes	30	80
fruit and berry squeeze	100	150

ANALYSIS OF THE BASIC TRENDS OF OBTAINING PROTEIN FROM THE WASTES OF THE VEGETABLE PROCESSING INDUSTRY

G.G. Mikeladze

There are two main trends in resolving the problem of protein deficiency. The first is to increase the productivity of the biosphere, and the second to produce biosynthetic protein.

At the present stage of development in science and technology, the way to increase resources of protein from nontraditional sources may be to obtain protein concentrated products and isolates from various cereals and plants containing protein. For this purpose waste materials from harvesting and the products of secondary processing of materials of vegetable and animal origin may be used.

Intensive work is being carried out all over the world, directed towards this end, i.e. increasing the useful productivity of the biosphere in respect of protein. In many countries there is a large tonnage production of protein from soy beans, and the production of protein from seeds of rape, sunflowers, sesame, clover and ground nuts, as well as from the green parts of plants is also taking place.

A considerable proportion of vegetable proteins are used in forage, but in the near future the percentage used in food will increase and this will improve the overall efficiency of their use.

Taking into consideration the soil and climatic conditions of Georgia, it is very important to produce leaf protein. For the production of leaf protein the green parts of various plants may be used as raw material. The type of plants can be divided into four groups.

To the first group belong the creeping plants, which are used as forage. To the second group belong plants that are cultivated for grain production. To the third group belong vegetable plants, such as spinach and sorrel. To the fourth group belong the leaves of plants qualified as by-products. These are the leaves of plants such as the tomato, carrot, radish, beet, potato, pumpkin plants etc.

Other possible sources of protein are tea and grape vine ends. We are carrying out investigations to produce various fractions of protein-vitamin concentrates without any residual waste material. Two fractions are obtained:

1) a cyto-plasmic fraction containing 80-85% protein, of a light grey color, having no smell and taste, which is proposed for use as food.

2) a chloroplastic fraction containing 40-45% protein, of a green color, with a specific grasslike smell, rich in vitamins, characteristic for each type of plant. Its proposed use is as a forage addition, but the same plants can also be used as vegetable meal. After the above separation, the waste material is used to produce microbial protein.

The most effective way of producing protein from raw material resources is by microbiological synthesis, as microorganisms develop quickly and in the productivity of protein synthesis they exceed animals and plants a thousand times. Besides, another advantage is that their cultivation on various substrata is possible.

Parallel to the raw materials we have in the form of stocks of oil, gas and others, microbial protein may be synthesized from ligno-cellulose substrata, of which there are large and stable resources, as they are replaced every year.

The question of the usage of microbial protein in food is at present not yet resolved as in many cases it has not been tested sufficiently to satisfy the health regulations.

Research work is being carried out in this field, such as for example the extraction of protein, so that in the selection of nonpathogenic microorganisms and substrata for their cultivation, the product does not contain substances harmful to man's health. Thus microbial protein comparable with vegetable protein may be considered one of the main resources for meeting the deficit of food protein in the future.

To resolve this problem the primary task consists of establishing the supply of stable raw materials which exist in nature and are replenished every year. The establishment of raw material supplies for the production of food proteins does have some difficulties, as an important part of the prospective raw materials are waste materials or secondary products from the processing industry.

Each of them at present has a definite place in the whole ecological system. Therefore if we do not work out some measures for preventing an infringement of the balance in the ecological system, caused by use of a given raw material, we cannot count on it as a stable raw material resource for obtaining protein.

The other important task towards the solution of the protein problem consists of making available technology and equipment for its large tonnage production. This technology must not cause a rise in the price of the product nor require a greater expenditure of energy.

The proteins thus obtained must satisfy the conditions required of proteins used for food and forage purposes in their amino acid composition, in the absence of toxic compounds and rheological properties.

To obtain microbial protein yeast, bacteria, and moulds are used. Not long ago yeast was considered to be the most advantageous. However the attitude to them as producers of protein changed because of some defects characteristic of these microorganisms. The main objection to them is the high content of nucleic acid in the biomass. Besides, most of the yeasts cannot use polysaccharides for their vital activity and we must cultivate them on preliminarily hydrolized substrata which requires considerable energy and financial expenditure. These defects may be avoided to a certain extent by obtaining proteins of fungal origin.

Mould fungi, as protein producers, differ from the other microorganisms by a much higher content of amino acid. Nucleic acids are 1.5 - 2.8% in fungi, whereas in the biomass of yeast their quantity reaches 12% and in bacteria 10-16%. Such a low content of nucleic acid allows us to apply the fungal protein in unlimited quantities. A distinctive feature of microscopic fungi is their ability to assimilate the most varied substrata and to accumulate biomass of full nutritional value. Yeast grows only on monosaccharides, the fungi grow on polysaccharides such as starch, cellulose, hemicellulose and even lignin.

Microscopic fungi synthesize a large variety of enzymes. This enables them to change the waste material of agriculture and food production into edible protein without preliminary hydrolysis. As a result of the thorough analysis of different ways of protein biosynthesis by microorganisms, the most effective and economic way is considered to be the cultivation of nonpathogenic strains of mould fungi on substrata not containing components which are harmful to one's health. The purification of protein isolates from harmful components passed on from substrata or synthesized by microorganisms is out of the question. Such purification is not often possible as it causes a rise in price of the products and may reduce the food value of the protein itself.

To get microbial protein we studied all the prospective sorts of waste material and secondary raw materials from the food industry and agriculture of Georgia. We also studied their

chemical properties and conditions to be applied for use as a substratum for the biosynthesis of protein. When we chose the microorganisms we took into consideration the ability of the raw material to enable synthesis of the protein to take place without particularly complicated preliminary processing.

Waste materials from wine-making, the canning industry, brewing, flour milling, and waste material from agriculture (maize, cabbage stumps, rough stalks and heads of sunflowers, waste material from peas processing, etc.) were found to be very effective.

Depending on the peculiarities of the substratum and the specific character of fungal strains, we recommend different methods of cultivation. For waste materials with a high content of cellulose and lignin, the technology of surface cultivation is used. For liquid waste materials or for waste materials which contain a lot of pectin and which are heated during sterilization, we apply the method of submerged cultivation in the liquid phase.

We have worked out a technology for obtaining a protein-enzyme complex (PEC) using the method of solid phase fermentation which produces material with a crude protein content of 25-30% and which has high cellulase activity.

Tests of PEC in poultry farming showed that forage, made on the basis of PEC, excelled a control forage in all indices (weight addition by more than 46%, requirement of PEC forage for 1 kg live weight less than 35%, and the test animals had a high resistance to infection). We have worked out the technology of obtaining protein isolates from PEC obtained by both surface and submerged culture. The study of the amino acid composition of the protein isolates showed us that proteins containing essential amino acids and obtained by these methods meet FAO requirements and surpass the proteins of oil-bearing and many other plants.

After carrying out tests for the safety of the product the protein isolates will be used to raise the biological value of some food products. Preliminary experiments have shown the possibility of their use in confectionary, in bread products, and in dairy and meat products.

Thus, the occurence of waste materials and secondary products in large quantities in the world provides supplies of raw materials which are replenished every year. Protein production from these nontraditional sources presents a good opportunity for creating new steady supplies of food and forage protein.

UTILIZATION OF CELLULOSIC WASTES FOR THE PRODUCTION OF FODDER YEAST AND/OR ETHANOL

G. Nagy, R. Kerekes, P. Somogyi,
J. Rezessy-Szabo, and B. Vajda

Up to the present there have been two main trends for the utilization of cellulosic wastes, namely:

a) the combustion of wastes to obtain energy, and
b) fermentation processes.

Single cell protein, liquid and gas energy resources, bioactive materials and chemical basic materials can be produced by fermentation. The production of microbial proteins and chemical compounds as energy resources are the most important, as there is a considerable shortage of these materials. Known fermentation technologies are expensive and their application is limited to specific types of wastes.

Our aim was to develop a technology which can be used for different kinds of wastes. Wastes are mainly lignocellulosic in type. The decomposition of natural cellulose and lignin is a relatively slow natural process. The microbial destruction of cellulose has been studied intensively, but less is known about lignin.

Our first task was to find a suitable microorganism for the fermentation of different kinds of wastes. As yeasts are widely used as a protein source in fodder, we prefered to use them instead of bacteria, and thus eliminated the problems which might emerge in the use of bacteria for feeding. We succeeded in isolating a special kind of Candida utilis strain which can be applied to specially pretreated cellulosic wastes. This strain was isolated from rotting wood. Its generation time is practically identical (G=1, 3h) to that when either glucose or cellobiose are used. This value increases by 30% on a carboxy-methyl-cellulose substrate (G=1, 7h) and by 50% on a Macherey-Nagel MN 300 cellulose powder substrate.

As waste materials from plants do not contain cellulose in a freely usable form, they must be pretreated to make the cellulose usable so that it can be fermented by our strain. For this purpose we elaborated a method based on the combined use of moderate heat and chemical treatment. Acid hydrolysis of cellulose has been described as a process where the terminal glucose units cleave step by step. When studying the decay rate of cellulose treated with diluted acid, we found that not only glucose molecules but also cellobiose and cellodextrin of different molecular sizes were present in the reaction mixture. The dynamics of this process showed that destruction of the cellulose molecule also took place within the molecule. If we treat cellulosic wastes with warm, dilute mineral acid (about 0.4 w/w %), decomposition can be observed. The cleaved cellulose reacts simultaneously with lignin and other plant components. Optimal solubilization depends on the acid concentration, the applied temperature gradient, the reaction time and the nature of the waste used. The solubilization process can be described by non-linear Hamilton equations and has to be controlled by a computer.

Our technology is based on the simple treatment of decomposing cellulose to make it fermentable under use of our strain.

Cellulosic wastes are cut into pieces of 5 cms by means of a chaff-cutter. The cut material is put into the digestor, where it is mixed with dilute mineral acid. Heat treatment is controlled by means of a non-linear temperature gradient according to the computer program described above, keeping the final temperature below 100^{0}C. So the process can be carried out by atmospheric pressure. The solid part of the mixture - mainly lignin - must be separated on a vacuum drum filter and can be utilized as an energy source for the fermentation process.

The culture medium for fermentation is made from the liquid phase with the addition of inorganic N and P salts and by adjusting the pH using slaked lime. Otherwise fodder yeast production is carried out by conventional technologies.

Our method can be put into practice using existing fermentation plants equipped with a chaff-cutter, a digestor and a filter. It is important to note that in this case a molasses-sterilizer and a clarifying separator are not necessary. The process does not produce more waste as the solid part can be used as an energy source after pretreatment and the fermentation liquor can be used as an agricultural fertilizer after the yeast cells have been harvested.

Fodder yeast can be produced by aerobic fermentation and ethanol by anaerobic fermentation with good yields using our Candida strain.

About 50-60% of dried fodder yeast and 60-70% absolute ethanol can be produced depending on the total carbohydrate content of the waste. The quantities of fodder yeast or absolute ethanol produced are shown in the following table.

Utilized wastes	Dried fodder yeast (kg)	Absolute ethanol (l)
	produced from 100 kgs of dry waste	
Corn stalk	11.5 - 16.5	14.2 - 19.6
Wheat straw	12.6 - 17.2	15.4 - 20.2
Wastes of sugar-cane harvest	18.0 - 19.0	22.0 - 23.6
Kenaf waste	16.0 - 18.0	20.0 - 22.0
Molasses (using conventional technology)	24.0 - 26.0	30.0 - 33.0

The cost of the production of fodder yeast produced by our technology meets the cost of soybeans generally used as a protein feedstuff. As the cost of production depends mainly on the cost of waste transportation, it can be reduced considerably if wastes are used in their place of origin.

A patent for our process is pending; patent rights therefore prevent us from publishing further details at present. If you would like further information, it can be obtained from the authors directly.

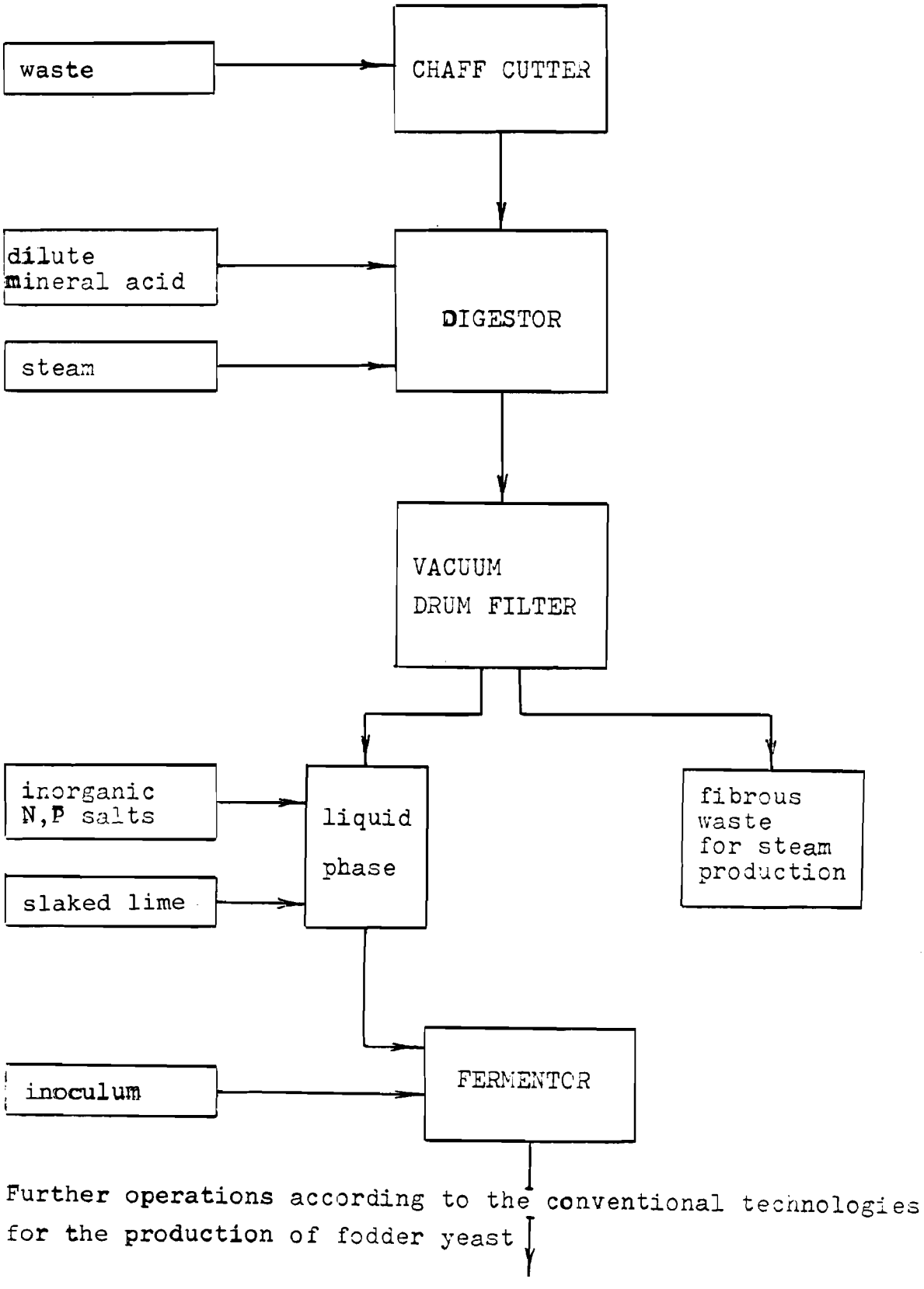

Figure 1. The steps of the process

THE UTILIZATION OF LIGNO-CELLULOSIC WASTE MATERIALS

J. Holota, and P. Belianksy

The population of our planet is increasing at a faster rate than our food resources, especially the energy and protein giving components. If we do not work out some convenient technologies for the utilization of non-traditional raw materials, it might be a matter of just a few decades before we are faced with a serious shortage of food supplies. Many scientists are paying great attention to the problem nowadays.

In Czechoslovakia research has concentrated mainly on the problems of manufacturing energy and protein giving food components based on non-traditional raw material resources. In theory, some suitable materials are available, for example: synthetic alcohol, n-alkanes, lignocellulosic waste materials, etc. Waste materials which contain saccharidic components and which might be reutilized are sulphite-spent liquor, and molasses. The two latter mentioned materials are already commonly used for fermentation, but their quantities are limited by new developments in cellulose and sugar manufacturing technologies. Excrements might be utilized for hydrolysis and when detoxicated used for direct feeding or for fermentation. However, the protein yield is negligible and the economy of the operation dubious. Synthetic alcohol is an excellent raw material for protein manufacture. The specific yield is high and no waste water is produced. But this process also has disadvantages: 4 tons of petrol are needed to manufacture 1 ton of protein. Manufacturing costs are therefore very high. Besides, synthetic alcohol is an exactly defined chemical compound much needed in other more important industries. Synthetic alcohol is manufactured on the basis of imported and strategic raw materials. Microbiological transformation of n-alkanes in protein is promising as a process, but at present the carcinogenity of the product has not as yet been established and the costs of installing such a plant are comparatively high. Manufacture is based on imported raw materials and supplies can not be guaranteed in the long run.

The remaining resources are lignocellulosic materials, so-called biomass, which have the following advantages:

-- biomass is the most abundant of all organic materials on earth, and even oil, coal, and possibly some minerals such as calcium carbonate might originate from it. Biomass is a renewable material and approximately 85 milliard tons is produced yearly.

-- some lignocellulosic waste materials from the wood processing industry are favorably located and therefore transportation fees are limited.

-- their chemical composition is very similar and except for a few special cases they are not toxic.

These properties make the use of lignocellulosic waste materials the most promising non-traditional resource for the manufacture of protein and energy giving food components.

In theory there are many methods of utilising lignocellulosic materials. Taking the situation in Czechoslovakia we decided to include the following four methods in our research program:

1. The transformation of sawdust into bulk fodder as a substitute for hay, clover, etc.

2. The hydrolysis of lignocellulosic waste materials and the fermentation of hydrolysates for protein production.

3. The evaporation of hydrolysates and the preparation of sugar concentrates as substitutes for molasses.

4. The microbiological utilization of wastes of the lowest quality, including lignin from hydrolysis for soil fertilizer, as a substitute for manure.

In the first case our research program was based on the observation that sawdust from beech, birch, poplar trees, etc. is very similar to hay, clover and other traditional fodder materials in chemical composition, but its digestibility is substantially lower. Significant differences can already be noted in lignin components. It was later proved that, not the quantity of the lignin, but the way it bonds the saccharidic components, causes wood to resist microbiological degradation, much as in nature or in the digestive tracts of ruminants. Our task was to find a treatment which loosens the bonds between the lignin and the saccharidic components of wood, resulting in exposure of the saccharides to the effects of microorganisms in the digestive tracts of ruminants. The effect of many chemicals has been tested combined with thermal treatment to this end. We finally came across combinations which gave rise to an increase in digestibility of beech sawdust from an original level of 4-5% to 55-58%. In order to test digestibility we improved the "in vitro" method, which enables between 50-70% of the samples to be tested in 3 days and obtains results corresponding to those gained by the

"in vivo" method. The treated sawdust was tested in large scale feeding trials with yearling bullocks over a period of 160 days. In comparison a second group of bullocks were fed on a standard diet. Results were very promising. No adverse effects were noted and weight increases were favorable.

One advantage of treated sawdust is that it is an excellent absorbent of various fodder components, e.g. minerals, antibiotics, etc. It is also very suitable for granulation, pelletization and thus may create a good basis for the manufacture of a standard and uniform fodder.

An area in which treated sawdust can be applied is in the feeding of wild animals in the forest and the zoo. For this purpose treated sawdust is mixed with other fodder components and pressed into large granules. The results of these experiments were good and the damage to young trees was substantially reduced.

The same treatment can be applied to other lignocellulosic materials, for example, straw. Orginal digestibility of about 40% may thus be increased to 60-65%.

From the technological point of view it is possible to increase the digestibility of all lignocellulosic materials to the level of digestibility of bleached chemical pulp, e.g. to 85-90%, but the increase in chemical cost and technological expense is very high.

Despite all these positive results the technology described has not yet been put into practice. No equipment is sufficiently developed for a continuous technology and the economic effects are disputable, because the price of raw sawdust is high.

The second method of utilizing lignocellulosic materials is hydrolysis. There is a large choice of catalysts and technologies resulting in the same final products, e.g. solutions of sugar, so-called hydrolysates, and solid waste, so-called lignin. Different acids, alkalis or salts may be used as catalysts in hydrolysis. The price and hydrolytical activity of catalysts however limits the choice. Of the acidic catalysts, sulphuric acid and hydrochloric acid are commonly used. Alkalis used as catalysts not only cause hydrolysis and the dissolution of sugars, but dissolution of lignin as well. Additional separation of these two components is difficult and costly. Therefore, these methods, despite higher sugar yields, will most likely not be put into practice. Of salts, superphosphate is used as a catalyst by the French Agrifuran method. The big advantage of this method is that no anticorrosive metals are needed to build reactors, and the solid waste can be used as an excellent soil fertilizer, because of its high phosphate content. The yields obtained from hydrolysis are, however, low and the economy of the process is debatable.

Methods of acid-catalyzed hydrolysis are, at present, the only methods which have been tested in large-scale manufacturing processes. During World War II there were some hydrolysis plants

in use in the USSR, Germany, Switzerland, Korea, Japan, the USA, and so on. In the boom period after the war, however, all these plants, except those in the USSR, were closed down because they proved unprofitable from the economic point of view.

The oil crisis and the increasing shortage of some important raw materials has altered the situation and research has recently been more concentrated on the problems of using lignocellulosic waste materials.

At the Forest Products Research Institute (SDVU) in Bratislava we too have formed a team consisting of chemists, biologists, designers, engineers, etc. Using previous knowledge as a starting point, we began by building a hydrolysis plant for continuous operation with a capacity of 5 kg/h dry weight of sawdust. In the meantime we tested various domestic sources of lignocellulosic waste material in experiments on the lab scale in sealed glass tubes.

The plant for the continuous process was built over a period of 3 years and has already been completed. Continuity of flow through pre-heated and heated tube reactors is achieved by high pressure using gas bombs and the loading is regulated by a discharge valve. The operating temperature is kept at 240^{0}C, pressure at 4MPa, and transflux is 0....50 liters per hour. We aim to use this plant to optimalize technological regimes of hydrolysis of various lignocellulosic materials, to prepare sufficient quantities of hydrolysates for fermentation experiments, and to concentrate it for feed trials. Because of a shortage of time, we have not managed to carry out many experiments using this plant yet and as yet no final results are available.

The experiments that were conducted and completed were correlated on plots as shown (see Figs. 1-6). The results of bark hydrolysis are of special interest. According to published data the addition of bark to wood chips for hydrolysis is limited to]0%, but no explanation is given for this. It is true, that bark has a lower specific sugar yield than wood, but because of its greater weight, the total sugar yield obtained from the operation is approximately the same. Another reason for this limitation might be the lower ability of fermentation of bark sugar because of the higher polyphenolic content in the bark. In preliminary experiments the adaptability of the microorganisms was, in fact, very low. However, further experiments conducted with already adapted strains of microorganisms (Candida utilis) were successful. The biological purification of the waste waters of bark hydrolysates after fermentation is unproblematic. By chromatographical analysis 28 phenolic spots (mostly unidentified) were found in the bark hydrolysates. Sixteen of these were assimilated during fermentation and 8 during the purification of the waste water. This means that the polyphenolic components in bark do not hinder its use in hydrolysis.

The highest sugar yields obtained by hydrolysis were from agricultural lignocellulosic wastes, especially from corn cobs. The yields from bagasse are of special interest because they are unusually high despite the fact that bagasse is a waste material of cane-sugar manufacturing.

In Figures 1-6 the sugar yields obtained by a two-step hydrolysis of various lignocellulosic materials under different conditions is illustrated, with:

the 1st step of hydrolysis being at 130°C, 60 mins.
the 2nd step of hydrolysis being at: a = 220°C
b = 200°C
c = 180°C
d = 160°C
concentration of H_2SO_4 is: 10,2 mml/100g for: a
5,1 " for: a, b, c
2,55 " for: a, b, c

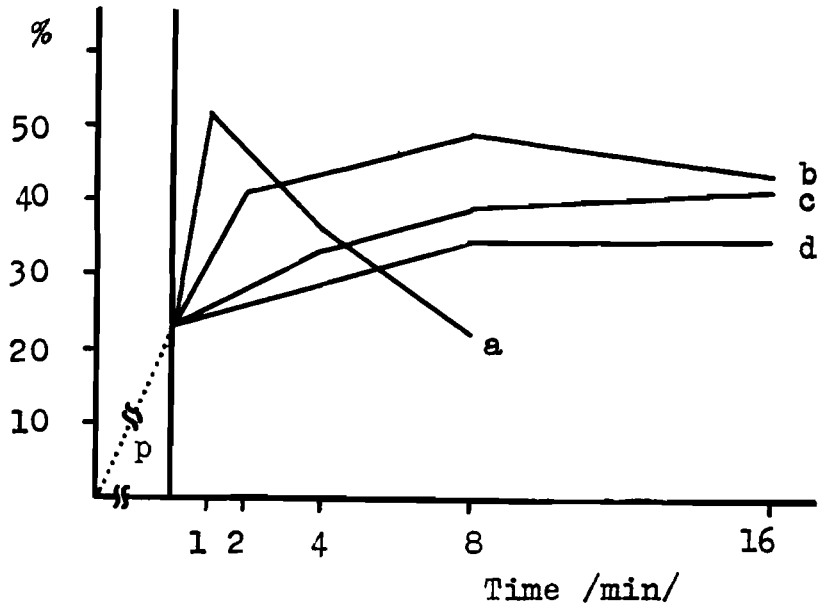

Figure 1. The Hydrolysis of Wheat Straw

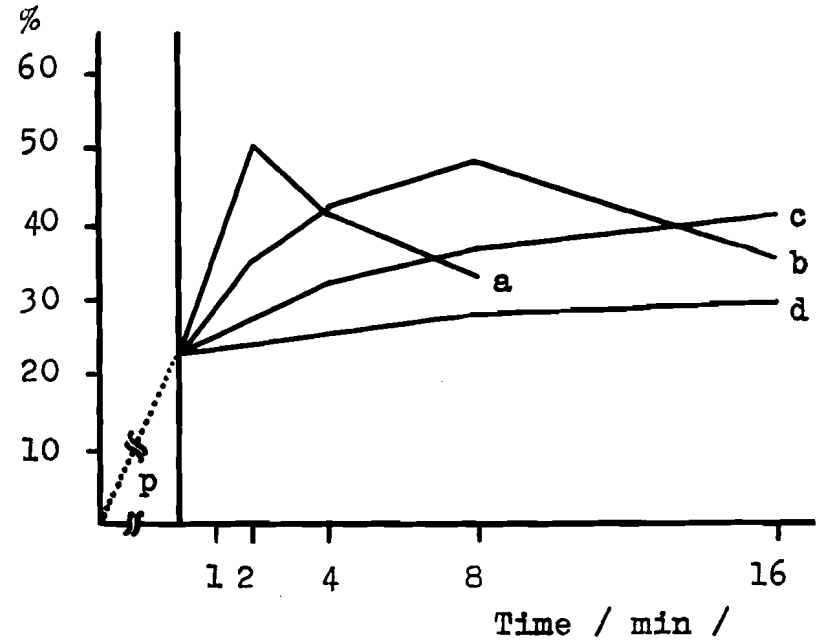

Figure 2. The hydrolysis of beech sawdust

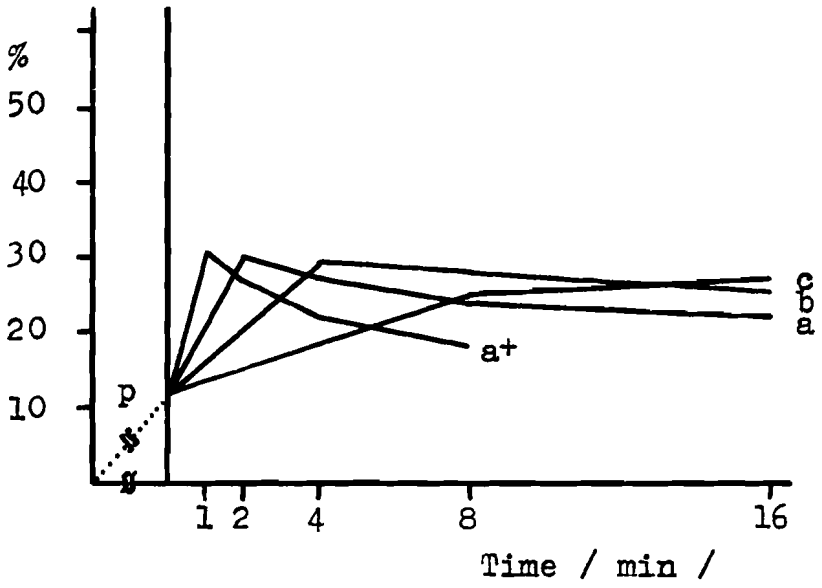

Figure 3. The hydrolysis of beech bark

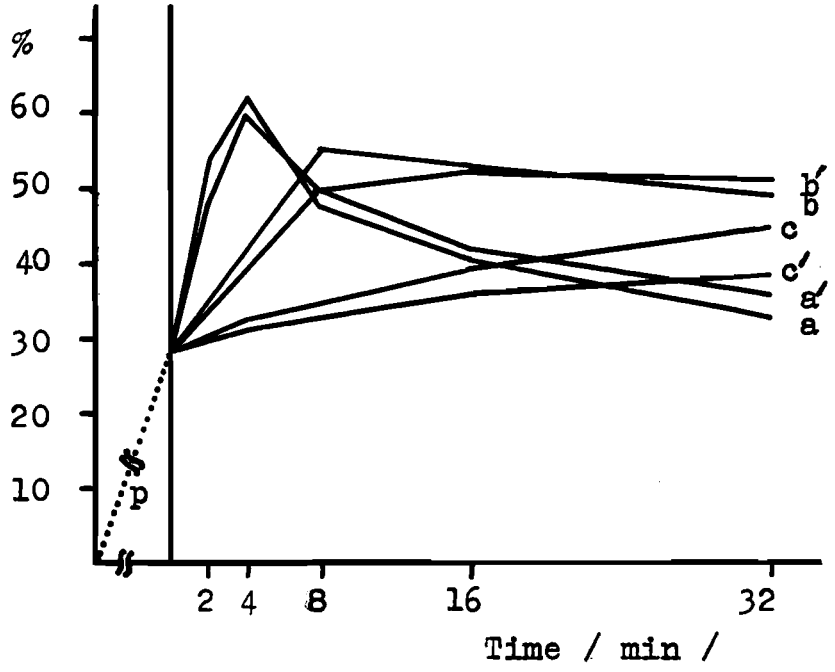

Figure 4. The hydrolysis of bagasse

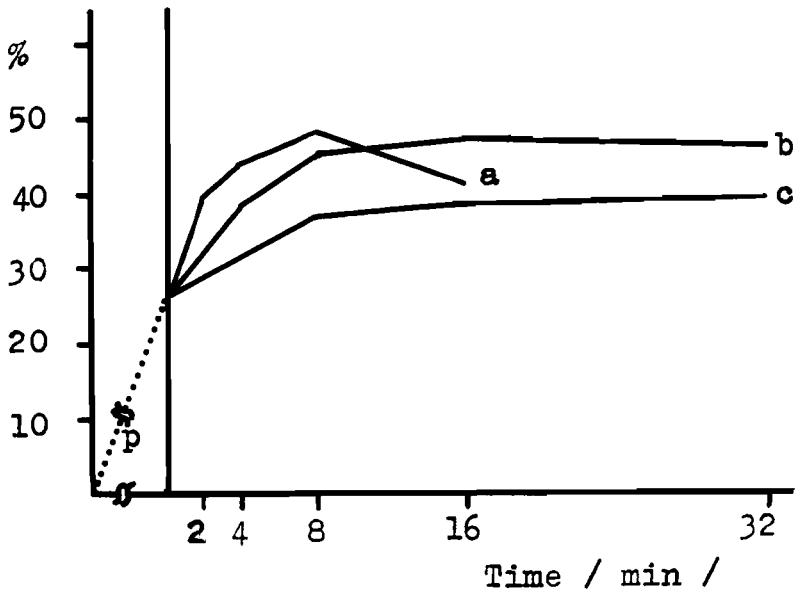

Figure 5. The hydrolysis of sugar cane leaves

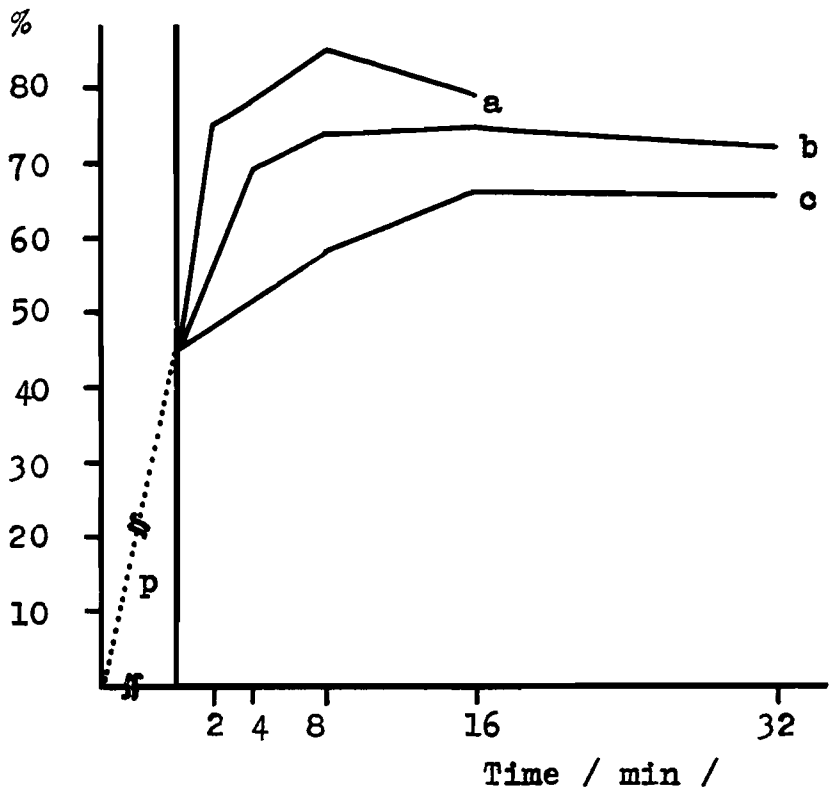

Figure 6. The hydrolysis of corn cobs

Our main interest lay in the hydrolysis of wheat straw because large numbers of hydrolysates are produced and they have no appropriate use. Another advantage of a straw-based hydrolysis plant is that this material has already been handled in the pulp and cellulose industry. Available equipment for the transportation of straw, its storage, loading of the digesters and, of course, the know-how to handle these difficult operations are of value when starting a new production.

Our program was made up of small scale experiments with a few grams of milled material, then experiments with kilograms of chopped straw and finally batch experiments using 100 kgs of straw. Hydrolysates from these experiments were used for fermentation. The solid waste was used as a substrate for the cultivation of edible mushrooms (Pleurotus ostreatus) for food, while the secondary wastes were successfully fed to ruminants.

Hydrolysates were neutralized before fermentation with lime milk to a level of pH 3,5 at 95°C, then with ammonia liquor to a level of pH 4,5 at 75°C. After decantation the hydrolysate was treated in a column filled with activated charcoal at a high temperature. It was proved that the charcoal column could be regenerated indefinitely using 2% of NaOH and 2% H_2SO_4. After this treatment the hydrolysate was a colorless liquid and no traces of furfural could be detected. We carried out some screening tests on various microorganisms and the best strains were used for our large scale tests. The best results were achieved using Candida robusta at the first step and Paecilomyces variottii Bainier 9N* at the second step of fermentation. The yields obtained in a continuous fermenter (based on the sugars used) were 63% biomass, and the sugar concentration decreased from 3,21 to 0,27%. The protein content in the biomass was approximately 53%.

The above results obtained from hydrolysis are relatively satisfactory. We have developed a new method of hydrolysis based on treatment of sawdust with hydrochloric gas in a fluid reactor. The advantages of this method are:

- -- no high temperature and pressure is needed;
- -- the distribution of the catalyst on large surfaces of wood is easy and homogeneous
- -- no additional devices are required for transportation of sawdust
- -- the time of reaction is short
- -- the sugar yields are very high
- -- hydrolysates of any concentration of sugars may be produced
- -- hydrolysates do not contain products of degraded sugar or toxic materials, such as furfural, oxymethyl furfural, etc.
- -- the catalyst can be regenerated.

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* Fermentation experiments were conducted at the VU-LIKO Institute (Research Institute for Distillery Effluents) Bratislava, where the above strain of Paecilomyces was selected and described.

However this method also has disadvantages, for example:

-- a high rate of corrosion of hydrochloric gas, especially when HCl is regenerated
-- all the materials and the whole plant have a stifling and irritating smell
-- difficulties of transporting and preparing hydrochloric gas

A small fluid reactor (3000 ml) was built at our institute with an adjustable gas circulation and a built-in cooler. Hydrochloric gas was brought from a large chemical factory in rubber containers. Beech sawdust sorted through sieves and impregnated with HCl was put into the reactor, which was later filled with hydrochloric gas. Then fluidisation was started using a built-in ventilator, and the circulated gas cooled by a built-in cooler. The speed of rotation was not measured. The temperature was kept at a level of 20^0C. After 5...120 mins of reaction the fluid was discharged through a sampler, then exposed to a short thermal shock, diluted with water and extracted. Finally the hydrolysate was inverted. When the initial moisture content of the sawdust was 30%, approximately 60% of the HCl was absorbed during fluidisation, but the final concentration of HCl in the sawdust made up approximately 45%. After applying thermal shock (at 170^0C, for 10 mins) the sugar yield was between 60-63%.

The third way to utilize non-traditional lignocellulosic materials is to manufacture thick wood-sugar concentrates, i.e. so-called wood molasses. A similar material is Masonex, a by-product gained by wood defibration using the Masonite process. This method is mostly used in the USA, and Masonex has high sales there, because of its high energetic value.

In the CSSR we produce two similar products in small quantities by causing the waste water to evaporate from fibre board manufactured by the wet process, and causing pre-hydrolysate evaporation from beech wood from pulp and cellulose manufacture. Both of these products are used successfully in animal fodder.

The conversion of hydrolysate into similar sugar concentrates is just a matter of economically evaporating excess water. We therefore do not expect any additional problems to arise concerning the preparation of similar products based on the hydrolysates of any lignocellulosic materials.

The fourth and last method included in our research program is the manufacture of soil fertilizer from the solid wastes of hydrolysis and other degradable organic wastes, e.g. bark, excrements, various sludges, etc.

Lignocellulosic materials are degraded by microorganisms to humus naturally. However under natural conditions this decomposition takes a long time. Our task was to speed up this process. We achieved this by adding balanced portions of mineral fertilizer. This did not require any microbiological initiation, since a sufficient quantity of microorganisms is

present in bark to start the decaying process. The time of decomposition lasted approximately 3 months. The decomposed material was then analyzed and chemical methods of indicating when a sufficient degree of decomposition has been reached were found.

The composted material was tested in large scale field trials using various agricultural products. The addition of compost has a long-term influence and the best harvest was achieved after 3 years' of application.

The big advantage of the above method is that it does not require large investments, expensive machinery or exact technology. Because of this fact, small scale plants can also be operated economically.

The methods of utilizing lignocellulosic wastes described above are, of course, just a few of the possible examples of using these materials as substitutes for other materials which are scarce or in short supply. But extended financial support and cooperation amongst many professions is needed in order to be able to consider all these possibilities, and no one country can afford this today. Therefore the best solution would be to make this project part of an international program under the supervision of some organization such as the FAO.

ENZYMATIC DEGRADATION OF PLANT SOURCES AND PROTEIN BIOSYNTHESIS THROUGH MICROORGANISMS

C. Panayotov, I. Stoyanov, T. Nikolov and K. Markov

The production of economically feasible, and biologically acceptable, single-cell protein is a basic problem of modern microbiology. The existing methods adopted using yeasts, bacteria and algae on different raw materials are complicated and, consequently, to a considerable degree, energy-consuming and much more expensive than the widely used traditional plant proteins.

Fungi have long been neglected as sources of protein, mainly because of a lower reproduction rate. However, they do possess advantages which have only recently started to attract attention. These are: a considerably more powerful cellulolytic system which degrades part of the cellulose itself, digesting it to protein without using any other carbon sources; the presence of an insignificant amount of nucleic acids in the end product; a better composition of aminoacids, etc.

Table 1. Enzyme Activity (Zone diameter in mm)

Strain	24 H	48 H	72 H
Aspergillus	19.0	18.0	18.0
Penicillium	21.0	19.0	16.0
Cephalosporium	16.0	15.0	15.0
Trichoderma	23.0	22.0	22.0
Alternaria	18.0	17.0	17.0

Our method uses different strains of thermophilic and other fungi, which degrade and digest cellulose of some plant by-products (corn stalks, etc.), and simultaneously accumulate protein concentrations in a single-stage process.

Table 2. Hydrolysis of Cellulose to Glucose

Strain	24 H	48 H	72 H	in mg % total sugar G/400g raw material
Aspergillus	347	252	284	42.5
Penicillium	418	216	246	51.2
Cephalo-sporium	145	92	91	34.4
Trichoderma	636	348	369	58.6
Alternaria	326	187	192	40.1

The fermentation conditions were chosen in such a way that, beginning with the preliminary preparation of the raw material, the substrates, regime and equipment are as simple as possible, the time as short as possible, energy requirements at a minimum, and the final product of a high protein content.

Table 3. Raw Material Transformations (g/100 g raw material)

Strain	O H		24 H		48 H		72 H		End percent of cellulose assimilation
	Cell.	Lign	Cell.	Lign	Cell.	Lign	Cell.	Lign	
Aspergillus	32.4	24.2	23.6	24 2	18.4	28.1	16.2	24.0	50
Penicillium	32.4	24.2	26.4	24.2	21.2	24.2	18.4	24.1	43
Cephalosporium	32.4	24.2	28.2	24.2	25.7	24.2	21.7	24.2	33
Trichoderma	32.4	24.2	20.4	24.1	17.3	24.0	15.4	23.9	53
Alternaria	32.4	24.2	27.2	24.2	26.1	24.2	20.3	24.2	37

In our studies, a cellulose degradation of about 50% was achieved and a concentrate with a crude protein content of 21.2% was obtained in a 48 hour fermentation. This is about twice the quantity which modern yeast protein production obtains from vegetable by-products, while energy requirements remain low.

Table 4. Protein Yield (g/100 g raw material)

Strain	24 H	48 H	72 H	End percentage in relation to cellulose by 48 H
Aspergillus	12.2	19.0	17.1	19.0
Penicillium	9.3	18.2	15.0	18.2
Cephalosporium	6.1	12.4	9.5	12.4
Trichoderma	14.2	21.2	18.4	21.2
Alternaria	7.4	14.1	11.2	14.1

Conclusion

Results of our preliminary experiments on the saccharification of cellulose suspensions by crude cellulases were comparable to those obtained by other investigators. It has been shown that yields above 50% makes the process attractive and economically feasible on a commercial scale.

ON THE PROSPECTS OF CELLULOSE BIODEGRADATION IN AGRICULTURAL AND FOOD INDUSTRY WASTES

S.V. Durmishidze, and G.I. Kvesitadze

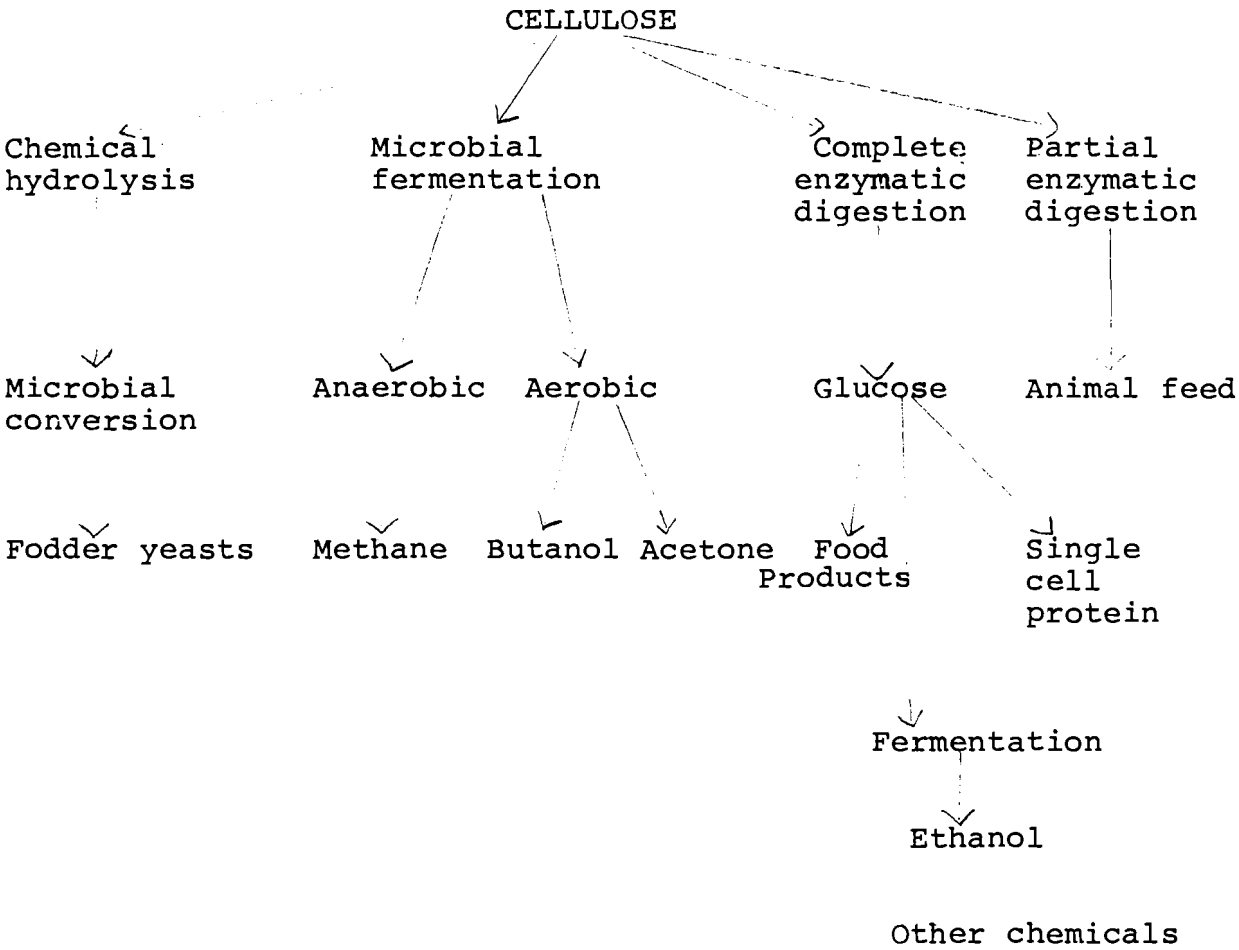
Cellulose - the most widespread compound in the organic world is an annually renewable resource (more than 150 billion tons) and can be considered to be almost an unlimited energy source. The possibility of converting agricultural, industrial, and urban wastes to useful products (glucose, ethanol, fodder yeasts, etc.) has stimulated the growth of new branches of technology for the conversion of cellulose substrates.

In addition, the idea of producing glucose suitable for use in food products from the wastes of the food industry is also interesting and it is known that glucose can be obtained by the hydrolysis of starch by special enzymes (amylases). However, results recently obtained at the Institute of Plant Biochemistry of the Georgian Academy of Sciences show that some of the cellulose wastes of the food industry can be used successfully to obtain food glucose enzymatically. This is possible because of the lower lignin content in the substrates refered to below; it is the presence of lignin which creates one of the main difficulties in cellulose bioconversion.

A number of agricultural wastes, such as maize stumps, sunflower stems and heads, the green leaves and shoots of vines, tea plants and the wastes of several other plants comprising hundreds of tons can be considered as prospective substrates for obtaining food glucose.

The value of this kind of work is increasing because of the possibilities of reprocessing wastes of the food industry and agriculture, involving the extraction of proteins, organic acids, carbohydrates, and other valuable food compounds, or in some cases compounds valuable for their biological activity.

A diagram of all possible conversions of the products of partial or complete hydrolysis of cellulose, is given below:



The main aims of our present investigation are:

1. to reveal the prospective cellulose substrates in wastes of the food industry and agriculture;

2. to select thermophilic microorganisms which are active producers of cellulases which have a high thermostability;

3. to elaborate an economical technology of enzymatic hydrolysis of cellulose wastes to glucose;

4. to evaluate various wastes of the food industry and agriculture in the Georgian S.S.R. for cellulose and lignin content. On the basis of this data the most suitable substrates will be selected and the technology of their conversion to glucose investigated.

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SELECTION OF FUNGAL SPECIES AND SOME EXAMPLES OF INVESTIGATIONS ON THE PRODUCTION OF FUNGAL PROTEIN

J.T. Worgan

INTRODUCTION

Fungi in the form of the macro fruiting bodies (sporophores) gathered from the wild have probably been eaten by man since prehistoric times and more than 2000 edible species are reported in literature. Microfungi have also been consumed in appreciable amounts in food products such as cheeses and in the fermented foods which are a substantial part of the diet in oriental countries. It is however only in recent times that it has become technically feasible to produce fungal mycelium by factory processes. Details of the processes for producing mycelium as a protein sources were given at the Task Force Meeting held at the International Institute for Applied Systems Analysis in Laxenburg, Austria in September 1980 and have been reported in the Collaborative Paper published by the Institute (ed. J. Hirs, 1981). Only a brief summary will therefore be given in this paper.

PROCESSES FOR FUNGAL PROTEIN PRODUCTION

Fungi grow in the form of individual hyphal strands which matt together to form a cohesive biomass known as mycelium. In the submerged culture method mycelium is dispersed in a liquid nutrient medium provided with an aeration system and with the pH and temperature controled at the optimum for growth. Growth by this method as rapid and extensive as that for the established process for yeast production has been shown to be feasible for a number of fungal species.

An alternative method involves inoculating an open structured solid substrate such as straw through which the mycelium penetrates as it grows. Growth by this method is much slower and the product

is liable to have a much lower protein content (Worgan, 1978a). Unchanged raw material used as substrate is difficult to separate from mycelium and has therefore to be part of the food or feed product. The main advantage of the method is that simpler equipment is required and the capital costs of establishing a process are less than those for the submerged culture method.

CRITERIA FOR SELECTING FUNGAL SPECIES

Fungal species suitable for the production of protein for livestock feed or for food products should have the following characteristics:

1) Nutrient requirements - grow on media prepared from readily available low cost materials.
2) Growth and product yield - grow rapidly and extensively and give good yields from the raw materials supplied.
3) Protein content - yield mycelium with a high protein content.
4) Protein quality - the mycelial protein should be of good nutritional quality.
5) Non toxic - the mycelium should have no harmful effects when consumed by livestock or humans.
6) Acceptability - when incorporated in livestock rations the presence of mycelium should not reduce the feed intake. For human consumption flavor and texture are important factors.

Within a species differences in some of these characteristics occur between different strains. Protein quality will probably be an invariable property of the species.

1. Nutritional Requirements

The main chemical elements essential for microbial growth are listed in Table 1. Although for the optimum growth of fungal mycelium there is some quantitative variation between the requirements of species the values reported do give an indication of the order of magnitude. Minor quantities of trace elements may also be needed and are usually present in sufficient amounts in materials from biological sources. The fungal species selected should obtain all the elements except carbon from simple inorganic compounds available as bulk chemical products and in particular should be able to use ammonium salts or urea as the nitrogen source. The carbon source is required in the greatest proportion and is provided from materials which are known as substrates. The cost of the substrate is a significant factor in determining the overall process cost and for this reason it is an advantage if waste products can be used. Molasses is one of the few wastes in which the C source is present in the form of simple compounds such as sugars. Most of the other waste products from agriculture, forestry or food processing contain a complex mixture of compounds

Table 1. Chemical elements required for the synthesis of microbial biomass

Chemical Element	Quantity
C	8.0
N	1.7
S	0.06
P	0.25
Mg	0.04
K	0.15
Ca, Zn, Fe, Mn	< 0.03

SOURCE: J.T. Worgan. Protein Production by Micro-organisms in Plant Proteins, edited by G. Norton. (1978, pages 191-203).

including natural polymers such as starch, pectin and hemi-celluloses. Many wastes also contain cellulose or lignocellulose. In order to convert these substrates quantitatively to mycelium the fungal species must be able to break down the polymers to simpler compounds which can be assimilated and used as nutrients for growth. The fungal species must therefore produce the appropriate enzymes to enable it to utilize all of the substrate.

If a fungal species shows any indication that it is able to use a substrate for growth then it is feasible that by adaption and by investigating the growth conditions that it can be induced to grow rapidly and extensively. This principle was applied to Fusarium Semitectum which initially gave poor growth on starch and on lactose substrates. Growth as rapid and extensive as that from glucose was eventually obtained from both substrates (Worgan, 1976).

Materials which contain the more resistant forms of cellulose or ligno-cellulose are exceptions to this principle and will require chemical or mechanical modification before rapid mycelium growth is feasible. Methods for reducing the resistance of lignocellulose to microbial attack were discussed at the Task Force Meeting in Laxenburg (**Worgan, 1981**). Some examples of investigations with the fungal species Sporotrichum pulverulentum on less resistant ligno-cellulosic substrates are given later in this paper. Fungi which produce the large fruiting bodies known as mushrooms do grow on cellulose and ligno-cellulose in their natural habitat. With a few exceptions most of the edible species studied have been found difficult to grow rapidly in submerged culture even when they are provided with easily assimilated sources of carbon (Worgan, 1968).

In some waste materials the presence of inhibitors of fungal growth may make them unsuitable as substrates for some fungal species. The presence of solanin in potato haulm for example was found to prevent the rapid and extensive growth of F. semitectum (Worgan, 1978b).

2. Growth Rate and Product Yield

A growth rate similar to that for the production of Food Yeast is advisable. If for example a fungal process requires twice the incubation period of that for yeast this would double the capital and operating costs per unit of output. A slow growth rate also increases the possibility that contaminating micro-organisms will grow more rapidly than the required fungal species.

The maximum growth of a fungal species can not be determined without an extensive investigation of all the variations in environmental conditions such as pH, temperature and aeration. Variations in the proportion of nutrients in culture media and the age and quantity of inoculum must also be investigated. Adaption to the substrate is another factor which can increase growth rate. The growth rate of Aspergillus oryzae on starch wastes for example was increased when the conditions for maximum amylase production were selected for preparing the inoculum instead

of conditions which gave maximum mycelial yields (Worgan, 1976).

That the edible macro fungi (mushrooms) are difficult to grow rapidly in submerged culture has been referred to above. However the mycelium of species of Morchella is grown by submerged culture on a commercial scale in the U.S.A. and in Bulgaria the mycelium of Cantharellus citrarius is reported to be produced in 100 m^3 fermenters (Torev, 1969).

Continuous culture although more difficult to establish from a technical point of view does have considerable advantages as a production system. The stability of the fungal culture under these conditions is a species (or strain) characteristic and therefore has to be taken into account in selecting species for this type of process.

If protein yield is the criterion which is to be used to assess the process then the period of growth may be less than that needed to give maximum mycelial yields. This aspect is discussed below.

3. Protein Content

The composition of mycelium varies extensively with growth conditions and the nutrients in culture media. Protein content is not therefore an absolute characteristic of a species although there will be a limit to the maximum protein content which is possible when the species is grown under ideal conditions. The proportion of carbon to nitrogen, the C:N ratio, in the nutrients provided is one of the most important factors and for most species is between 8 and 20.

The growth cycle of a fungal culture grown in a medium which is not limiting in nitrogen is illustrated in Figure 1. The protein content of mycelium harvested at (A) is approximately 50%, whereas at the longer period of time (B) it is only 30%. The protein yields are approximately the same. The time scale of the growth cycle will change with the temperature, pH, aeration conditions, nutrient composition and the age and size of the inoculum. All of these aspects and the time at which to harvest will have to be investigated before it can be established that a species will not yield mycelium with a high protein content.

Protein contents of mycelium are frequently reported as Total nitrogen x 6.25. That this can give misleading information as to the actual protein content of mycelium is illustrated in Table 2. Nucleic acid is included in the determination of Total N and is also a variable factor. The alpha amino nitrogen (AAN) x 6.25 is therefore a better measure of the value of mycelium as a source of dietary protein.

Figure 1. Growth cycle of a fungal culture

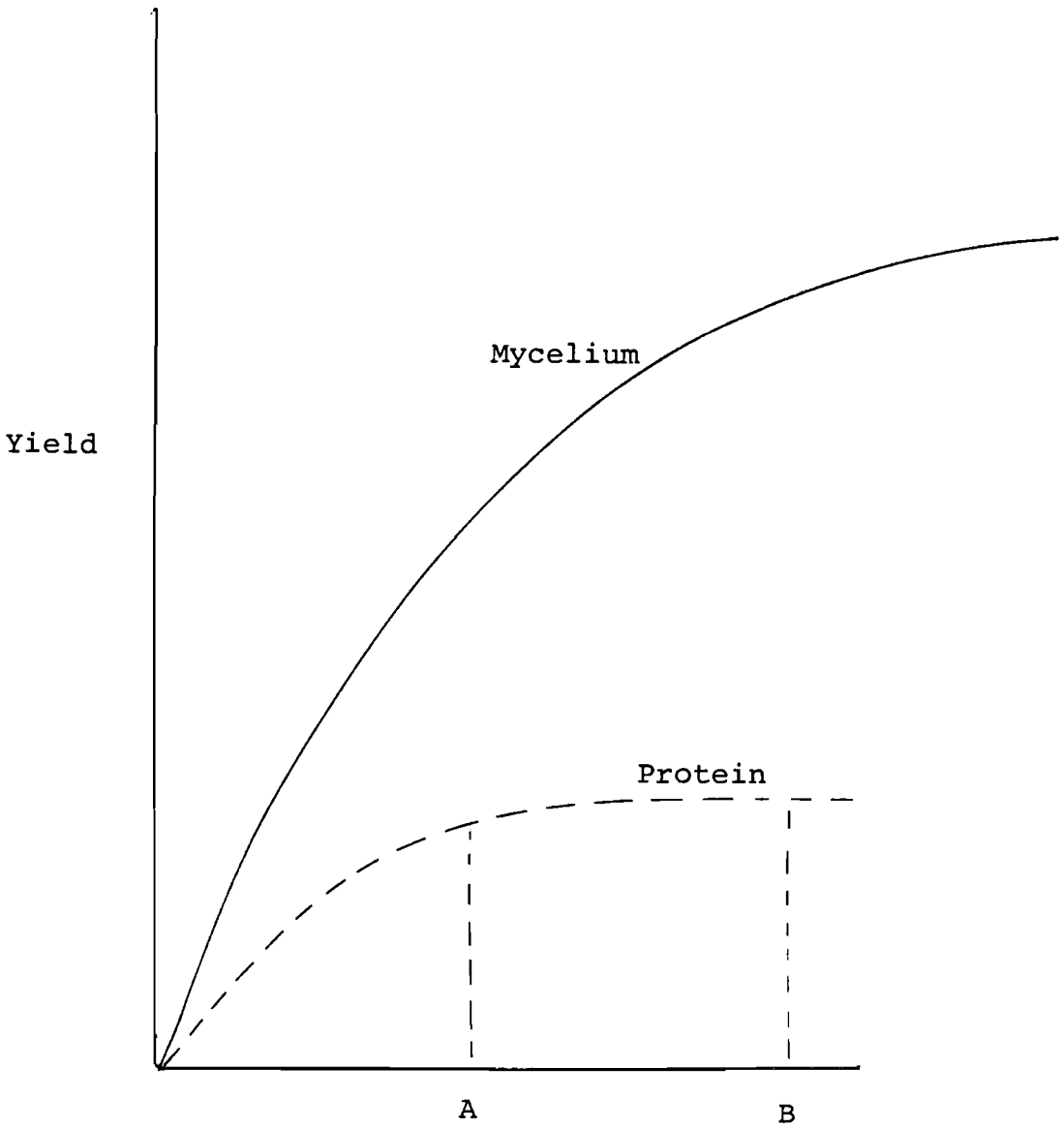

Table 2. Proportion of nitrogen compounds in the mycelium of F.semitectum

	% of dry mycelial wt	
	24 hr culture	96 hr culture
Total N	9.1	5.8
Chitin N	0.8	1.2
Chitin N as % of Total N	8.8	20.3

SOURCE: J.T. Worgan. Wastes from Crop Plants as Raw Materials for Conversion by Fungi. Pages 23-41. In Food from Waste.

4. Nutritional Aspects

That assessments based on the crude protein in mycelium (Total N x 6.25) will not give a true representation of nutritional value has been discussed in the paragraph above. Reports on analyses of the essential amino acids in the protein of mycelium indicate that most species investigated contain all the essential amino acids required by livestock and by human beings. The relative proportions of these essential amino acids do vary and in most species cystine and methionine are the ones which are often below the requirement specified for an ideal protein as represented by the FAO Reference Protein (FAO/WHO, 1973). An excess of lysine above the FAO requirement is an advantage because the mycelium can then supplement cereal rations in which lysine is usually the limiting amino acid. Some examples of the essential amino acid content of fungal proteins are given in Table 3. In the table, unless otherwise specified, all other essential amino acids are present in proportions equal to or greater than those in the FAO Reference Protein.

The digestibility of the protein in mycelium also influences nutritional value. Net Protein Utilization value (NPU) is the most useful assessment of the value of a protein source in the diet because the NPU measures both the digestibility and biological value of the protein source. Some examples of the NPU values for the protein of fungal species are given in Table 4.

Cystine, methionine and lysine are the essential amino acids most sensitive to heat and losses in nutritional value may occur during the drying process. This aspect should be noted when preparing samples for assessment. An improvement in the digestibility is a possibility if autolysis is allowed to occur before drying.

Although primarily considered in this conference as a source of protein, vitamins are also present in those species which have been investigated. Mycelium can also be induced to accumulate lipids although this reduces the protein content. The fatty acid composition of A. oryzae is similar to that of vegetable oils and it is possible that it could be used as a source of fat in the diet (Kauer 1981).

5. Safety Aspects

That the mycelium of some fungal species has been consumed extensively in food products over a long period of time was referred to in the introduction to this paper. In Japan, for example, nearly one million tons of Miso, produced by the growth of A. oryzae on rice, are consumed each year. This species and a number of others therefore have a long history of their extensive consumption without any apparent ill effects. The mycelium of fungi which have not been used extensively for either food or feed will require a thorough testing program before they can be used. Guidelines for a program of testing have been published by the Protein Advisory Group of the UN (1970).

Table 3. Essential Amino Acids in the protein of fungal mycelium

	g amino acid/100g total amino acids	
Fungal species	Lysine	Total Sulphur
F. graminearum [16]	7.5	3.2
F. semitectum [15]	5.7	2.7
A. oryzae [17]	7.2	2.8
S. pulverulentum* [11]	6.2	4.4
FAO Reference Protein [8]	5.5	3.5

* g amino acid/100g protein

SOURCES: 8 = FAO/WHO. Energy and Protein Requirements. Technical Report No. 522. 1973

11 = B. von Hofsten. Cultivation of a Thermotolerant Basidcomycetes on Various Carbohydrates. In Food from Waste, pages 156-166. 1976.

15 = R.H. Smith et al. Article in J. Sci. Fd. and Ag. 26:785. 1975.

16 = C. Anderson et al. In Single Cell Protein 11. Pages 314-329. 1975.

17 = T.W. Barker et al. J. Sci. Fd. and Ag. 32:1014-1020. 1981.

Table 4. Nutritional value of the proteins of fungal species - Nett Protein Utilisation (NPU) values

Fungal Species	Crude Protein Basis		Total Amino Acid Basis	
		Methionine supplemented		Methionine supplemented
F.graminearum [18]	46.3	62.6	59.9	84.7
F.semitectum [15]	42	66	59	92
A.oryzae [17]	65		80	

SOURCES: 15 = R.H. Smith et al. Article in J. Sci. Fd. and Ag. 26:785. 1975.

17 = T.W. Barker et al. J. Sci. Fd. and Ag. 32:1014-1020. 1981

18 = I.F. Duthie. In Single Cell Protein 11: pages 505-544. 1975

To follow the full program with the number of animals specified is expensive and requires a lengthy period of time. Preliminary screening tests are therefore advisable. Any indication of even a trace of adverse symptoms in these tests suggests that further investigations of the species will not be worthwhile.

Species on which tests have been made that indicate the mycelium and which will probably be safe to use as a food include:- F. semitectum (Worgan, 1976), S. pulverulentum (von Hofsten, 1976), and Geotrichum candidum (as referred to at this Task Force meeting by R. Marchant et al.). The mycelium of Morcella has been approved a food product in the USA (Litchfield, 1967) and Paecilomyces variotii (Romantschuk, 1976) as a livestock feed in Finland. Fusarium graminearum has probably been subjected to the most extensive testing program and is approved as a food product in the UK.

6. Acceptability as Food or Feed

From the reported results of feeding trials it is unlikely that the incorporation of mycelium into livestock rations will cause any problems. Exceptions will be species which produce bitter flavors. For use in products for human consumption flavor and texture are important. Unless a food can be produced from mycelium which people are prepared to eat the whole process is invalidated. One of the problems with Food Yeast is that it consists of a powder without functional properties and it is therefore difficult to produce acceptable food products.

Because of the presence of the hyphal strands matted together to form mycelium fungi do yield products which have characteristic texture. The mycelium of F. semitectum, for example, has a texture when chewed in the mouth similar to that of pressed chicken meat and to a limited extent this texture can be varied by growth conditions (Worgan, 1976).

Mushroom or nut like flavors are reported for the mycelium of several fungal species. Some of the cheese moulds and fungi from some oriental foods have unpleasant bitter flavors when grown separately as mycelium and this means that they would not be satisfactory as food products. A bland flavored product is preferable because suitable flavors can be incorporated to suit the taste of the consumer.

Conclusions on Species Selection

In a screening program for the selection of a suitable fungal species for the production of protein for food or feed it is advisable to carry out preliminary toxicity tests as soon as possible. Any adverse result means that the species would not be worth further study. It would also be advisable to assess the nutritional value by an analysis of the essential amino acids in the mycelial protein. The absence of an essential amino acid or a low value for the proportion present in the protein, relative

to that in the FAO Reference Protein, means that the mycelium will have a poor nutritional value. It is improbable that this characteristic can be improved by altering cultural conditions.

Growth rate, yield and protein content are not absolute characteristics of the species and can be improved by an extensive investigation of the environmental conditions under which the mycelium is grown. Even after an investigation program some species may give results which suggest they would not be worth considering for a practical process.

Examples of Species Investigated for Protein Production

All of the species listed below have to a limited extent satisfied the criteria discussed in the first part of this paper. Each has been tested for safety and shown no adverse effects. Only F. Graminearum, however, has had adequate toxicity trials to satisfy the safety regulations for use in human consumption. No species has been found to be perfect in meeting all the criteria which have been listed and no species has mycelial protein which is nutritionally equivalent to that of the FAO Reference Protein although most of the proteins are equivalent to soya. Nutritional information on the mycelium of the species is given in Tables 3 and 4.

F. semitectum - this species has been adapted to grow on lactose and starch substrates. On deproteinized cheese whey mycelium has been grown continuously for 1000 hours in a 25 litre vessel and on a starch waste has been produced in a 2,500 litre pilot plant. Feeding trials with mycelium as the sole source of protein in the diet have been made with rats as the test animals over a 2 year period through 3 successive generations. Tests made throughout according to the PAG Guidelines gave no adverse results. Feeding trials have also been made with pigs and poultry.

Investigations made on a number of liquid wastes suggest that this species does not possess as extensive a range of enzymes as A. oryzae and mycelial yields and reduction in the Chemical Oxygen Demand (COD) were somewhat less than those given in Table 5.

A. oryzae - the occurrence of this species in oriental foods has been mentioned above. The mycelium is grown on an industrial scale for the production of the enzyme amylase. Some results of investigations for the dual purpose of producing mycelium and at the same time reducing the polluting strength of effluents are reported in Table 5. Rapid growth and good mycelial yields have also been obtained on banana waste and citrus pulp substrates (Worgan, 1978b).

S. pulverulentum - this species does have enzyme systems which will degrade lignocellulose. Results of investigations on three fibrous wastes are reported in Table 6. In the case of sunflower heads and palm kernel meal the reduction in the cellulose content is sufficient for the product to be suitable as a feed for non-ruminant livestock. The lower cellulose and higher

Table 5. Mycelial protein yields and COD reduction from the growth of fungi on liquid wastes

Fungal species	Liquid waste	Protein yield g/l	Mycelial yield g/100g C source	COD reduction
A.oryzae	Palm [19]	11	56	91
	Olive [5]	10.5	-	-
	Starch effluent [3]	-	46	91
	Lucerne deproteinised juice [5]	12	-	68
F.semitectum	Palm [5]	9	-	85
	Citrus molasses [5]	9	-	-
	Lucerne deproteinised juice [5]	8.3	-	65

SOURCES: 3 = J.T. Worgan. In Food from Waste. Pages 23-41. 1976

5 = J.T. Worgan. In Nuevas Fuentes de Alimentos para la Produccion Animal (Spanish). Pages 304-335. 1978b.

19 = T.W. Barker and J.T.Worgan. European Journal of Applied Microbiology and Biotechnology 11:234-240. 1981.

Table 6. Conversion of Fibrous Wastes to Livestock Feed by S.pulverulentum

Type of waste	Composition - % of dry wt			
	Waste product		Waste product + fungal growth	
	Protein	Cellulose	Protein	Cellulose
Sunflower heads [5]	4.5	23	36.5	8
Waste cellulose pulp fibres [5]	0.4	74.5	31	9.6
Palm kernel press cake [20]	18.9	29.6	26.8	6.4

SOURCES: 5 = J.T. Worgan. In Nuevas Fuentes de Alimentos para la Produccion Animal. Pages 304-335. 1978b.

20 = Findings by E.K. Collison, 1981, unpublished data. Reading University, U.K., National College of Food Technology.

protein content of the waste cellulose fibres after mycelial growth does mean that the product could be incorporated into non-ruminant rations. The cellulose activity of the mycelium was developed by growing the inoculum by surface culture before proceeding to submerged culture for the production stage.

Feeding trials of the mycelium with rats as the test animals have been made and it has been reported that more extensive trials are to be undertaken (von Hofsten, 1976).

Paecilomyces variottii - this species is used in the Pekilo process which is in operation in Finland. The substrate is sulphite liquor, a waste from the manufacture of cellulose pulp. The process has the dual purpose of producing mycelium and reducing the pollution strength of the waste and is operated on a continuous culture system. The product is accepted in Finland for use as a high quality protein feed for livestock (Romantschuk, 1976).

Investigations on the species F. graminearum and Geotrichum candidum are reported in other papers presented at the Task Force Meeting.

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THE CULTIVATION OF MESOPHYLL AND THERMOPHILE FUNGI ON PLANT WASTES TO OBTAIN PROTEIN

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L.A. Zakordonets, and T.I. Bilai

Different microorganisms such as yeasts, bacteria, unicellular algae, actinomyces, and fungi are being studied as potential sources of protein.

Among protein producers fungi are remarkable for their components contain highly active enzyme systems and they are capable of utilizing diverse compounds including cellulose, hemicellulose and lignin. This makes possible the direct transformation (sometimes called dry fermentation) of starch and cellulose containing substrates to cell protein whose amino acid constitution corresponds to the physiological norm. The cells of the micelia contain far fewer nucleic acids and purines than those of yeasts, which is why the amount and the duration of the usage of the enriched food is not so limited.

The production of microbial protein on different wastes without hydrolysis is being investigated. A number of similar processes at the production stage include the extraction of the fungi protein "pekilo" from the liquid wastes of the paper industry in Finland, the fungi biomasses Penicilium, Fusarium, and Aspergillus on the wastes of the flour and potato industries in England. The mycoprotein from microfungal mycelium developed by the Lord Rank Research Centre in England has been approved by the government for test marketing as a human food, and the means to give it the structure characteristic of meat products are being investigated. Similar methods of obtaining protein are being studied in Canada with the thermotolerant fungus, Chaetomium celluloliticum, and in the USA on Dipiodia gossypina.

Ways of enriching roughage and the wastes of the agriculture and food industries with fungal protein are also under investigation. The careful selection of special strains of fungi is

important to the successful enriching of different substrates with protein. The problem of selecting new and effective nontoxic microorganisms which synthesize high quality protein is no less important than the task of choosing a rational technology and new sources of raw material for obtaining microbial proteins.

The object of our study was the cultivation of mesophyllic fungi (Fusarium and Acremonium spp. species) and thermophyllic fungi (Thielavia, Myriococcum and Absidia) which are known for their rapid growth and which create a considerable amount of biomass. The fungi cultures were kept in the museum of the Department of Physiology and Microsystems at the IMV of the Academy of Sciences of the USSR.

Taking into consideration the favorable prospects of using Hyphomycetes, a protein source, we conducted a comparative study of the ability of the Fusarium fungi species to synthesize protein on a substrate containing the husks of grapes and tomatoes, or wheat and rice bran as a source of carbon, i.e. residues received in large amounts, easily storable and transportable. We studied those strains having a rapid growth activity, protein accumulation and essential amino acids.

The protein content of Fusarium grown by submerged culture (72r) on a poor substrate such as Chapec was between 30.3-46%. The cultivation of Fusarium on plant wastes resulted in 2-3.5 times more protein enrichment compared to the control substrates (see Table 1). Fungi grew better on Chapec whereas the residues of grape and tomato squeezing turned out to be less suitable for protein growth and accumulation. As some substrates proved to be of little use for hypomycet growth, we combined different ratios of raw materials (see Table 2). The best combination proved to be that of wheat and maize bran at a ratio of 1:1. The use of the abovementioned combinations of substrates provides good aeration of the mixture, satisfies the different component requirements of fungi, and makes possible the production of protein of a high biological value.

Table 1. The enrichment of different substrates with protein of different strains of Fusarium

Species of fungus strain	protein content in micella on Chapec %	grape squeeze	tomato squeeze	wheat bran	maize bran
1	2	3	4	5	6
Fusarium spp.54260	41.9	25.6	-	27.9	20.8
Fusarium spp.2801	42.0	-	16.2	31.2	19.6
Fusarium spp.54258	33.0	-	17.5	35.0	20.6
Fusarium spp.54259	30.3	-	-	34.3	-
Fusarium spp.T	43.0-45.0	-	-	35.2	28.6
Fusarium spp.53211	35.0	-	17.5	29.4	20.1
Fusarium spp.	45.0	-	-	34.9	25.6
Fusarium spp.522	35.0	22.6	-	25.2	21.4
Fusarium spp.54257	30.3	-	-	17.3	-
Fusarium spp.5	42.0	-	-	25.9	22.3

Note: Here and in Table 2 "-" means that no measurement was made as bad growth was observed visually. The protein content in the control substrates was as follows: 4.6-6.8% in grape squeeze; 6-7.2% in tomato squeeze; 8.7% in maize bran; and 12.7% in wheat bran.

The above mentioned mesophyllic and thermophile fungi were grown on cellulose containing wastes of agriculture such as straw and cotton husks (Table 2). These strains were enriched depending on the species and strains of the fungus (from 3-4 to 7.8-16%).

Protein in fungi grown on different substrates contains an unusually high amount of amino acids including essential amino acids and vitamins (Table 3) which correspond to the technical requirements of protein products, after microbiological enrichment of agricultural wastes with protein.

Table 2. The content of crude protein in mesophyllic and thermophilic fungi after dry fermentation on different plant substrates (%)

Fungi	Straw	Cotton husks	Wheat bran	Wheat bran and maize from (1:1)	Wheat bran and grape squeeze (2:1)	Wheat bran and geranium wastes (1:1)
1	2	3	4	5	6	7
Mesophylls:	7.8	8.1	17.6	20.2	21.2	16.0
Fus.spp.						
54260						
53211	9.1	8.7	17.9	18.4	19.3	19.1
b/n	11.0	11.6	21.2	22.5	26.1	-
2801	12.0	10.2	17.3	18.7	21.8	20.2
54258	11.2	20.8	16.4	19.0	19.0	16.5
	12.0	11.5	20.2	22.2	25.9	-
51070	7.8	9.1	16.2	16.9	18.2	-
54259	8.9	7.8	16.5	17.2	17.9	-
522	8.0	9.2	19.4	17.5	25.6	-
	11.4	11.2	18.1	20.1	25.8	-
54257	8.0	8.2	17.3	18.1	18.6	-
Thermophiles: 6/IV	11.16	11.4	22.4	21.4	24.9	-
Absidia spp. 9/3	8.8	-	12.6	18.8	19.1	15.4
Thielavia spp. 62447	14.5	-	21.9	20.3	17.7	17.0
Myriococcum spp. 355	12.8	8.0	17.1	-	-	14.8
Thielava spp. 622660	16.5	15.5	-	-	-	-

Note: The fungi cultivation took 94 hours. The protein content in the control substrate was: in straw, and cotton husks 3-4%; in wheat bran, 12.7%; in wheat bran and maize bran with a ratio of 1:1, 10.8%; in wheat bran and grape squeeze, with a ratio of 2:1, 11.2%; in wheat bran and geranium wastes, 1:1 ratio, 9.9%.

Table 3. The essential amino acid and vitamin content in mesophyllic and thermophilic fungi grown on different cellulose containing substrates

Amino acids	100 grms protein	Vitamin of the product	(mkg)g dry
Lysine	6.1-6.2	Riboflavin	5.5-8.0
Methionine	1.0-1.3	Biotin	0.9-29
Arginine	4.6-5.9	Thiamine	2.0-4.5
reonine	2.8-5.3	Nicotinic acid	145-300
Valine	4.8-6.6		
Leucine	4.6-6.8		
Isoleucine	2.5-4.5		
Phenylalanine	0.2-1.2		
Histidine	2.2-3.0		

The above shows the prospects of using the method of solid substrate fermentation of starch raw materials and cellulose containing wastes of agriculture by means of hypomicetal fungi.

This will to a great extent, help solve the problem of utilizing the wastes of agriculture and some branches of industry along with the problem of protein deficiency.

OBTAINING A PROTEIN-ENZYME COMPLEX BY CULTIVATING MOULD FUNGI

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The production of microbial protein is one possible way of combatting protein deficiency. Microorganisms have a short development cycle and they are more productive than animals and plants.

Carbohydrates, mainly starch and cellulose, the supplies of which are replenished annually, are important raw materials for microbiological synthesis. Industrial wastes from the processing of raw materials can provide a source of carbohydrates.

The wastes and secondary products of the vegetable processing industry are important bioresources for the production of food and forage, especially when they are used as substrates for microbiological synthesis. The comparative stability of their chemical composition, the quantities produced, the lack of harmful components as well as other factors are the advantages of these resources which lead to their consideration as a base for industrial protein production.

In our studies on how to obtain microbial protein, we used fungi, because they have several advantages over other micro-organisms. Moreover, the content of nucleic acid in fungi is lower than that in yeasts. Because yeast protein lacks sulphur containing amino acids, the addition of these essential amino acids in order to increase the nutritional value is required, whereas when fungi are used this is not necessary. Besides, microscopic fungi have extensive systems of enzymes which allow them to utilize complex substrates during the growth process whereas yeasts cannot utilize these complex substrates.

The cell wall of certain strains of fungi is thin and can therefore be easily processed in the gastro-intestinal tract of humans and animals without preliminary processing. Animals

tested at different institutes throughout the Soviet Union and abroad have shown the safety of fungal biomass.

The Georgian S.S.R. has great reserves for microbiological synthesis of protein for food and forage. The most promising wastes and secondary raw materials for the production of protein in the republic are estimated to be 1809 hundred tons (gross weight), and 1010 hundred tons in a dry state (10-12% moisture) in 1985.

The following industrial wastes were used for protein biosynthesis: wheat and maize brans, grape and apple skins, wastes from the vegetable oil industry, whey, sunflower heads, etc.

We selected nontoxic strains of the mould fungus "Sporotrichum pulverulentum"from amongst the microscopic ones for the production of biomass with an extensive enzyme complex with a high protein content. The results of surface cultivation of the fungi on different wastes is given in Table 1.

Table 1. Results of surface cultivation of the fungi on different wastes

Raw material	moisture	crude protein	fat	ash	cellulolytic activity
1. grape skins + wheat brans	7.2	27.95	5.1	7.25	90.1
2. grape skins + apple skins	9.2	25.5	3.45	6.55	75.2
3. grape skins + distillery beer	11.0	26.0	4.4	5.65	72.1

From Table 1 it is clear that the best results were obtained when the fungus "Sporotrichum pulverulentum" was cultivated on a mixture of grape skins and wheat bran with a ratio of 1:1. Taking into account the above results, we continued our studies with this biomass.

The technological process of the protein-enzyme complex production by this method is given in Table 2.

The content of essential amino acids in proteins is the best indication of their food value for humans and animals. From this point of view our process is of great interest, because the product contains nearly all the essential amino acids. The high content of asparagine and glutamic acids should be pointed out

Table 2. The technological process of protein-enzyme complex production

Raw material	Initial moisture in the nutrient medium	Growth temperature	Growth duration (in hours)	Regulation of aeration m/h for 1 kg.
grape skins and wheat bran 1:1	55-60	28-30°C	72	8-12

as it is one more advantage of our process. Data of the amino acid content in the protein of the product are given in Table 3.

Table 3. The amino acid content in the protein of the product

Amino acid	content in protein %	FAO standard
1. isoleucine	2.1	4.0
2. leucine	3.69	7.04
3. lysine	2.17	5.44
4. methionine-cystine	1.78	3.54
5. phenylalanine + tyrosine	-	6.08
6. threonine	5.37	4.0
7. valine	-	4.96
8. arginine	3.03	-
9. asparaginic acid	24.13	-
10. serine	6.75	-
11. glutamic acid	31.15	-
12. proline	-	-
13. glycine	8.44	-
14. alanine	6.8	-

The content of nucleic acids in biomass is of importance. We determined the content of deoxyribonucleic and ribonucleic acids in the product by the Spirin method. The results are given in Table 4.

The content of nucleic acids is four times less than in yeast, and this fact proves the advantage of the fungal protein in comparison with yeast. The biomass obtained was tested by animal feeding trials at the Georgian Zootechnical-Veterinary Institute.

Table 4. Nucleic acid content in biomass

Biomass	nucleic acids	E_{270}	E_{290}	Content of the nucleic acids %
Fungus grown on grape skins + wheat bran	RNA	0.661	0.650	0.5
	DNA	0.762	0.732	2.0
			total	2.5

The results of testing showed the high feed value of the biomass. Increase in the weight of chickens in test groups was 46% greater than in controlled groups, and costs of the forage per kg of live-weight were 35% less.

According to preliminary calculations, production of the biomass is economically worthwhile. From the use of the whole quantity of wastes and secondary products available under conditions of large scale production, the annual economic effectivity is estimated at 22 million rub. per year.

The use of the protein- enzyme biomass in livestock farming allows us:

1. to make up the deficiency of protein in forage;
2. to use wastes and raw materials containing cellulose rationally; and
3. to enrich forage with vitamins and other biologically active substances.

THE TREATMENT OF DISTILLERY EFFLUENT TO YIELD MICROBIAL PROTEIN

R. Marchant, T.W. Barker, A.P. Murray, A.M. Patton, and J.P. Quinn

One of the most important distilled alcoholic beverages produced in the British Isles is whisk(e)y. During the production of Scottish and Irish malt whiskies approximately 90% of the fermentation volume remains after distillation i.e. 550 million gallons ($2.5 \times 10^6 m^3$) of liquid wastes are produced annually in the British Isles and must be disposed of by the manufacturers. Most of the volume consists of spent wash or "potale" - the primary residue left after the first distillation step - which has a biochemical oxygen demand (BOD) of as much as 50,000 mg l^{-1} and a pH of between 3.0 and 4.0. At present this effluent is disposed of by discharge to the sea, application to derelict land or after evaporation and mixing with spent grains (insoluble residue of the malt) is pelletted to form distillers dark grains. Distillers dark grains are then sold as a low grade additive to animal feeds. Each of these disposal methods has problems, since the spent wash is highly colored its discharge to the sea is often undesirable, its low pH is detrimental to plant growth and spreading on derelict land often leads to seepage into ground water systems. The presence of appreciable levels of copper and zinc in the wash also makes its long term application to land undesirable. When the dark grains process was introduced the economics of the process were favorable with energy costs low and feed prices relatively high. The situation is now rapidly changing and in the Old Bushmills distillery in Northern Ireland at least 25% of the total energy consumed is used in the dark grains plant.

It was against this background that we commenced our work to develop a biological treatment process which would act both as a waste treatment process and at the same time yield a valuable product, single-cell protein, which could be sold to off-set treatment costs. Any such system developed is in direct competition with the existing dark grains process and must therefore be more economically favorable.

Two main approaches are possible to the microbial treatment of a waste, to produce S.C.P; either to use the best already accepted organism or to select the most efficient utilizer of the available substrates and then to test the organism for its value and acceptability as a food source. The second alternative was the one we adopted. The spent wash from the malt whiskey production of Old Bushmills distillery, Co. Antrim, N. Ireland has a complex and variable constitution; the limits of variation of its constituents over a 2 year period are given in Table 1.

Table 1.

pH	2.9
Carbohydrates	6.7 - 21.2gl^{-1}
Protein	15.1 - 31.0gl^{-1}
Free amino acids	2.1 - 4.3gl^{-1}
Glycerol	4.4 - 7.5gl^{-1}
Total titrable acidity expressed as lactate	4.9 - 18.4gl^{-1}
Dissolved phosphorus	740 - 1570ppm
BOD	24500 - 43000mgl^{-1}

The selection of organisms by enrichment culture from the environment yielded three fungi each capable of rapid growth with high levels of substrate utilization on the spent wash. These were the yeasts Candida kruzei and Hansenula anomala and the filamentous fungus Geotrichum candidum. Although the whiskey production process is a batch one there are advantages in having the treatment process operating continuously, particularly for reduction of plant size and restriction of substrate variability. Continuous culture experiments have been carried out on the laboratory scale with the three organisms growing together in the bubble column type fermenter. BOD removal of up to 95% has been achieved without the addition of any nutrients or other modification of the feedstock. Due to the low initial pH of the wash the fermentation requires no aseptic procedures and the established mixed population of the three fungi resists external infection by other microorganisms.

Following the successful laboratory scale experiments air lift fermenters of 100 liter and 500 liter capacity were constructed to examine operational parameters and to produce biomass. The biomass for experimental use was harvested by centrifuging and was then spray dried, the analyses of product from a number of batch and continuous runs is given in Tables 2, 3 and 4. The crude protein content of the biomass is consistently 55% in continuous runs, but only 45% in batch runs.

Table 2. Biomass composition - mean values from 11 batch and continuous samples spray dried

	%
Moisture	2.0
Ash	6.3
Total lipid	2.2
Crude protein (N x 6.25)	48.9
DNA	0.4
RNA	8.6
Hexosamine	2.8
Total hexose	21.7
Available lysine	1.22
Copper	0.0112
Zinc	0.0286
True protein (by difference)	38.4
Calorific value	20.501 kJg^{-1}

Table 3. Fatty acid composition of biomass

	%
C14	2.8
C15	1.3
C16	25.3
C16:1	8.2
C17:1	1.8
C18	9.8
C18:1	20.8
C18:2	26.4
C18:3	3.8

Table 4. Amino acid profile of biomass $g(16gN)^{-1}$

	Representative sample of biomass	FAO reference protein	Requirement of growing rat	Requirement of pig
Phe	5.32	2.8		
Tyr	3.45	2.8	6.9	
His	1.92		3.5	
Ile	5.23	4.2	5.3	3.9
Leu	7.10	4.8	6.4	
Lys	6.35	4.2	5.3	4.8
Met	1.71	2.0	4.2	4.0 - 4.5
Cys	1.94	2.2		
Thr	4.68	2.8	4.3	2.9
Val	5.09	4.2	5.3	
Arg	4.61		1.8	
Try	not determined			0.9
Sum essential amino acids	47.39	31.4	44.0	
Asp	9.11			
Ser	4.51			
Glu	11.63			
Pro	5.05			
Gly	3.92	Available lysine 2.5		
Ala	5.84			

The nutritional and toxicological evaluation of the product has been carried out using rats and rainbow trout. The reason for using trout was to examine the possibility of substituting the SCP for expensive fish meal in the diets of farmed rainbow trout. Net protein utilization, digestibility and biological value for the SCP are given in Table 5 together with data for standard protein sources and dark grains. A further avenue explored involved mixing the SCP with spent grains in the proportions they would be produced and comparing this with the existing product dark grains. This new mixed product has approximately 5% more crude protein than dark grains and a biological value almost double.

Experiments with rainbow trout indicate that the SCP does not have a nutritional quality comparable with fish meal and indeed the rat experiments have shown the product to be deficient in sulphur-containing amino acids.

Table 5. Nutritional evaluation using rats

	NPU	Digestibility	
Casein	66	99	0.67
Soya	52	90	0.58
SCP	40	76	0.53
Dark grains	32	67	0.48
SCP + spent grains	55	72	0.76

A potential toxicological problem which was identified at an early stage was the presence of copper and zinc, leached from the copper stills and other plants, in the spent wash. This becomes concentrated in the biomass on occasion at levels up to 180ppm for copper although more frequently at lower levels (see table 2). Blood and organ analyses of rats and fish fed high levels of SCP for prolonged periods have failed to show any histological changes nor any disturbing concentrations of heavy metals in particular tissues. No significant effects have been observed in animals fed on acute test diets for 4 months, chronic test diets for 8 months or in teratogenicity trials extending into the second generation.

Pilot plant scale fermenters of 1500 liter capacity have now been constructed, but not yet operated at the distillery and a full scale process scheme is given in Figure 1 for a plant utilizing both the spent wash and spent grains to produce 3000-4000 tonnes of product per annum.

The prospects for the process appear bright at this time with a high quality product produced, we hope, at a cost less than dark grains. The plant processes are simple and are compatible with the technology already employed in the industry which is a traditional and highly conservative one. In addition the organisms used have a broad and flexible pattern of substrate utilization which could be exploited in the treatment of other distillery effluents or indeed in the treatment of many other low pH strong organic liquid wastes.

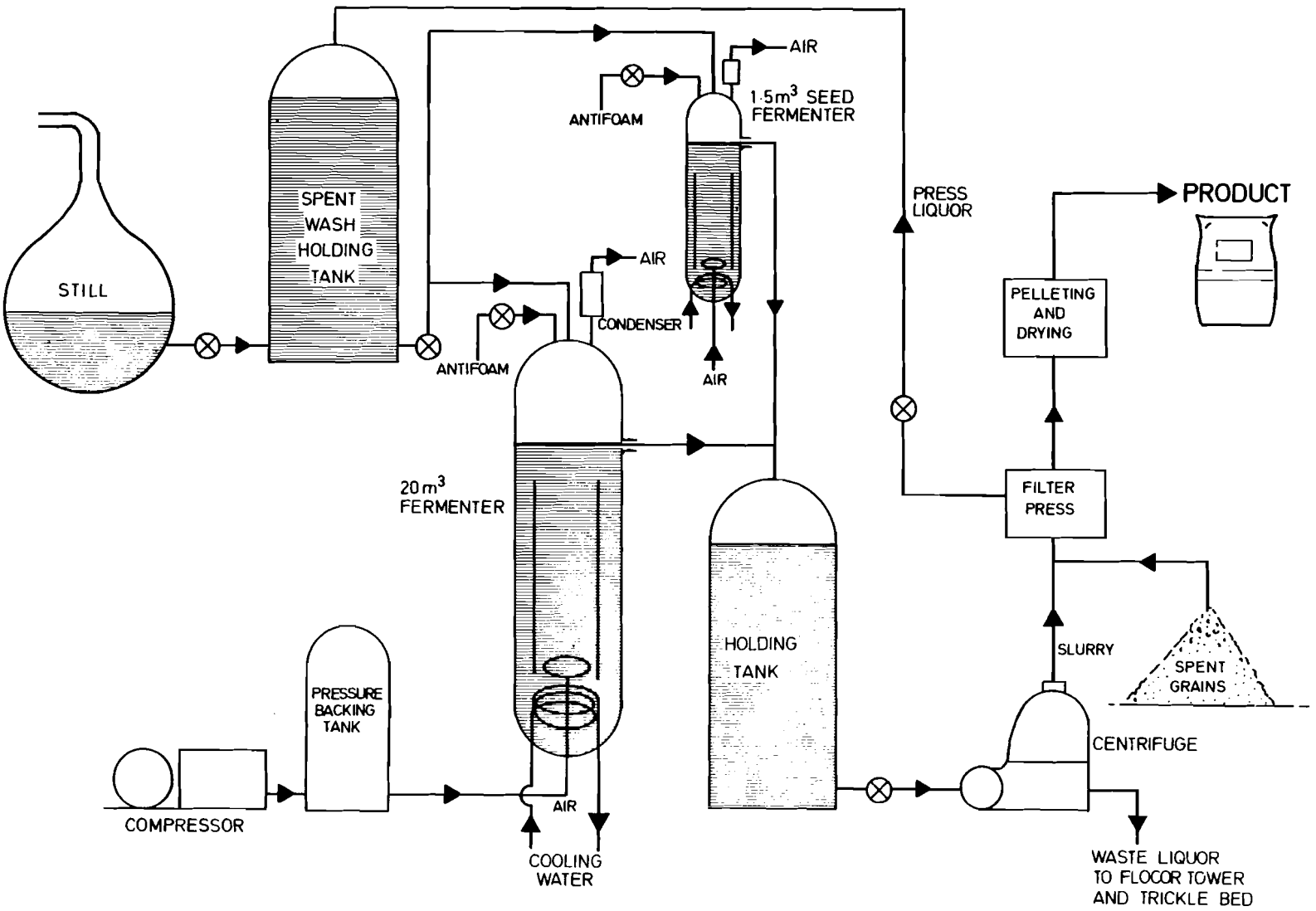


Figure 1. Flow diagram of the proposed treatment plant for distillery effluent

NEW HIGH PROTEIN FOOD BASED UPON THE FERMENTATION OF CARBOHYDRATE FEEDSTOCK

J. Edelman

Mycoprotein is a highly versatile food material containing nearly 50% protein and a high level of dietary fiber. Its RNA content is below the Protein and Calorie Advisory Group's guideline of about 2% maximum on a dry weight basis. The material can be either (a) converted directly from wet sheet filtered from the fermeter broth, and processed through the frozen or chilled food chain to foods resembling meats of various sorts, but especially white meats, or (b) it can be dried, stored indefinitely at ambient temperatures and later converted to foods or nutritional supplements.

The term mycoprotein denotes the origin from a microfungal mycelium. It is not a single cell protein (SCP) as it is not derived from a single cell organism and the program differs from SCP programs in being directed at the production of human food, not animal feed, and using food-grade carbohydrate as substrate and not hydrocarbon (e.g. gas-oil, paraffin) or hydrocarbon-based (e.g. methanol, ethanol) feedstock. Other main concepts at the start of the program were that the end products should be highly textured and delicious to eat, so that although they would be highly nutritious, the main driving force for their consumption would be their organoleptic qualities as food.

It was realized early in the program that continuous fermentation was a prerequisite for success. Many thousands of organisms were screened for their growth characteristics as well as end product qualities, which included high protein level with high biological value, long hyphae for texture, rapid and homogeneous growth, near theoretical yield from glucose, and stability over long periods of fermentation. The organism chosen was a strain of Fusarium graminearum. Over the past several years we have achieved successful fermentation runs of 1,000 hours or more in strictly aseptic conditions in the pilot plant

at High Wycombe using a 1300 l fermenter. The residence time in the fermenter is about five hours and gives rise to a slurry of fungal mycelium in the fermentation medium which leaves the fermenter continuously at a concentration of about 1.5g/liter. The level of RNA is reduced from about 10% to under 2% by a simple heat treatment in a stirred tank reactor which inactivates the proteases in the hyphae but which allows the RNAases to hydrolyze the RNA to monomers which diffuse out rapidly through the cell walls. There is a concomitant loss of other low molecular weight compounds from the cell, which imposes a penalty of some 30% loss of total dry matter.

The major problem of regulatory clearance by national Governments was evident from the start of the program and toxicological testing was initiated over ten years ago. At that time there were no protocols for the testing of a new food as against a food additive and the program broke new ground on several fronts. A battery of tests on animals was undertaken including acute toxicity, life span studies, teratology, and multi-generation fertility studies, in addition to nutritional evaluation (PER, NPU, BV) in animals and man, including human clinical tests and observations, and finally allergenicity studies in man. This program led to a two million word submission to the UK Government authorities, and in 1980 resulted in clearance for the use of the material in all applications for test marketing purposes under normal commercial conditions (it was agreed that until more experience has been gained in the market-place, mycoprotein will not be used in infant foods). Development of products for test marketing is now in progress, and the first examples of these new foods will be put on limited sale later this year.

The process is a much more economic means of converting an agricultural commodity, i.e. starch or sugar, to high added-value (e.g. "meat-like") food than any known animal route. Mycoprotein itself is a highly versatile ingredient for food manufacturing purposes: in concept it would be delivered as a consistent raw material to food processing units for conversion to a wide variety of foods. These could be convenience foods as eaten in affluent countries, albeit at lower cost and higher eating quality than many existing foods, and they would find their natural level in the market-place. In less affluent but emerging countries which have an accessible source of carbohydrates but insufficient animal protein foods to satisfy the market or sociological demand, mycoprotein could be used either in the wet or the dry form as high value foods or as nutritional supplements, e.g. in biscuits, breads or porages. It is not envisaged, in the first phases of industrial development of mycoprotein, that it would be produced in those poor countries where there is an insufficiency of food as a whole; in these cases it would evidently be more logical to continue using agricultural commodities, already in short supply, directly as a food source.

PRODUCTION AND USAGE OF THE AM-50 PROTEIN-VITAMIN CONCENTRATE FROM WASTE PRODUCTS OF THE FOOD AND LIGHT INDUSTRIES

B.G. Ordzhonikidze, and G.A. Tsilosani

The fodder products of the Georgian S.S.R. are known to contain insufficient amounts of digestible protein. Studies aimed at providing this lack of protein consist of a search for new sources of protein and also for limiting amino acids.

In order to provide for the shortage of protein in the feed of agricultural animals a new efficient method for the microbiological production of fodder protein from waste products has been suggested. Work was begun in 1975.

The microbial protein-vitamin concentrate was obtained from hide scrapings, the waste products of the leather-making industry, and from the dregs of beer breweries, for use as a substrate for the reproduction of microorganisms.

A Candida species was chosen from 15 strains of microorganisms supplied by the Institute of Microbiology of the Academy of Sciences of the U.S.S.R. to obtain mutants for protein and group B vitamin production. The AM-50 protein-vitamin concentrate was produced by inoculating the above mentioned waste products with the mutants obtained.

Experimental results were confirmed by practical application of the AM-50 protein-vitamin concentrate production method at the Akhmeta biochemical plant, which normally produces a protein-vitamin concentrate from liquid paraffin. According to the technological specifications of the feed grade yeast Candida guilliermendia obtained at the plant, the concentrate is characterized by a number of disadvantages: it is fire and explosion hazardous (both as a raw and a finished product); the dispersed paraffin is harmful for the personnel as it irritates the mucous membrane of the respiratory tracts; it consumes too high amounts of mineral salts and water; the Candida guilliermondia

strain becomes highly allergenic under certain conditions; and, finally, paraffin is known to be a carcinogenic substance.

In the method suggested here for the production of the AM-50 protein-vitamin concentrate, the nutritive medium does not require any preliminary sterilization or addition of mineral salts and water; it is cheaper than the one currently utilized at the Akhmeta biochemical plant. The final product thus obtained is actually a dry powdered biomass (see Table 1).

It has been found that, in the AM-50 protein-vitamin concentrate, the amount of the deficient sulphur-containing amino acids - methionine and cystine - is higher than standard requirements, the lysine content is twice as high as the standard value, and other essential amino acids are plentiful (see Table 2). Also, significant amounts of such vital elements as manganese and copper have been found in the concentrate. Furthermore, the microorganisms of the Candida genus were found to synthesize group B vitamins, such as ergosterol (provitamin D) and a number of other enzymes. The final product of the AM-50 protein-vitamin concentrate contains 50-60% of crude protein.

The AM-50 protein-vitamin concentrate has proved to be nontoxic. It was tested at the Georgian Research-Training Zooveterinary Institute, and at the Georgian branch of the All-Union Research Institute for Mixed Feed Production where it was tested as a fodder additive for chicks and young pigs. The test results were all positive.

The AM-50 concentrate has been tested for a number of years as a pollen substitute as an additional stimulant for feeding bees. Test results were quite successful and the Scientific-Technical Society of Apiculture acknowledged them in its recommendations. The economic effect of using AM-50 concentrate as a pollen substitute (1 kg of the concentrate for one bee family) with carbohydrate paste is 19.75 roubles, which means 1,398,250 roubles for apiculture throughout the whole Republic.

The microbial protein hydrolyzates can be successfully used as component parts of food concentrates and in tinned food. The food industries are expanding rapidly each year, and therefore produce greater amounts of waste. Utilization of this waste in the above mentioned way will enable a reduction in production costs and will make more complete use of raw materials.

Table 1. Properties and characteristics of the AM-50 protein-vitamin concentrate

Properties	Characteristics
1. Appearance	Powder
2. Color	Yellow or light-brown
3. Taste and smell	
4. Moisture content, %	8
5. Crude protein, %	58-60
6. Fat, %	4.0
7. Ashes, %	3.0
	The final product of the AM-50 protein-vitamin concentrate is not combustible and does not make an explosive mixture when exposed to air.

Table 2. Amino acid content of the AM-50 protein-vitamin concentrate

Amino acid	Raw protein content, %	
	AM-50 protein-vitamin concentrate	Standard value
Arginine	6.4	6.6
Histidine	7.8	2.4
Lysine	15.2	7.0
Leucine	6.9	9.2
Isoleucine	5.0	7.7
Phenylalaline	4.2	6.3
Threonine	6.5	4.3
Methionine	3.0	4.0
Cystine	2.4	9.2
Valine	7.8	7.2
Tryptophan	-	1.5
Glycine	7.13	5.2

THE UTILIZATION OF MICROBIALLY TREATED WASTE PRODUCTS OF AGRICULTURE AS PROTEIN SOURCES

D. Beck, Th. Kreuter, M. Ringpfeil, and K. Kehr

THE PROBLEM

Liquid mixtures of faeces and urine with water are waste products which are obtained in very large quantities and local concentrations especially from large-scale plants of industrial livestock production.

In trying to reduce environmental pollution and recycle secondary products, the aerobic microbiological conversion of these liquid wastes is becoming increasingly important. The development of ecologically and economically relevant technologies for the solution of these tasks is characterized by a trend towards highly productive automated plants where the amount of waste exceeds 1000 m^3/d. Due to the imbalance in the ratio of C, N and P in the substrate it is necessary to add secondary substrates in the form of highly concentrated, industrially available external carbon sources such as methanol in order to attain high efficiency in microbial conversion. This promising concept was described by M. Ringpfeil at the previous Task Force Meeting (ed. Hirs, 1981).

Large quantities of liquid-waste biomass are already being obtained in the existing sewage-treatment plants of industrial livestock production. This biomass could be used as a substitute for high-grade protein to feed livestock or grow mushrooms on an industrial scale. In this connection we have investigated the possibility of effectively using these protein sources which are of inferior quality compared to those used in other processes (Ringpfeil et al., 1980) (Beck et al., 1978). See Table 1.

Biosludge of this origin caused justifiable doubts concerning its suitability for recycling for livestock feed as it is a multicomponent mixture of an insufficiently controllable consistency

Table 1.

System	Crude protein % dm (N x 6,25)	Total nucleic acids % dm	Lysin g / kg dm	Ash % dm
Strain MB 58 / MeOH	>75	9,6 - 11,0	36	4,5 - 5,5
Strain MB 58 / MeOH Liquid wastes	72	7 - 10,5	32 - 40	4,5 - 5,5
Biosludge without external C - source	40 - 60	6 - 9	10 - 25	15 - 30

and composition. It contains soluble or finely dispersed calorifically and materially unused portions of the feedstuff, a considerable amount of inorganic salts and an excess of the aqueous phase.

Experiments with biosludge feeds have been carried out in several countries for a period of more than 20 years and have produced differing and contradictory results (Trjukenjo, 1979), (Thomanetz, 1978), (Jerock et al, 19). Therefore our aim was to find out which parameters of the biosludge obtained by the aerobic conversion of liquid wastes made a significant difference when compared to high-grade single-cell protein. See Table 2.

One should be aware of the fact that up to now many objections have been raised to the use of biosludge for livestock feed, because of:

-- the possible presence of pathogenic germs;
-- the inhomogeneity of the culture;
-- its high mineral content;
-- the imbalance of the essential amino acids;
-- its low digestibility and biological value;
-- its SO_2 content, and
-- its generally inferior qualities compared to those of microbial protein sources grown on pure carbon sources as well as its protein content and organoleptic features.

Table 2. Essential features of biosludge from liquid-waste compared to SCP from methanol

parameter \ biomass	biosludge from liquid waste	aerobic biomass from methanol
SO_2	0,95 - 1,5 %	0 %
Crude protein	40 - 60 %	75 %
Total nucleic acids	6 - 9 %	9,6 - 11,0 %
Ash	15 - 30 %	4,5 - 5,5 %
Lysin	10 - 25 g/kg	36 g/kg
Uniformity of the culture	mixed culture	≈100 %
apparent digestibility	53 - 63 %	84 %
Biological value	53 - 64 %	69 %
NPU	35 %	68 %

We examined the impact of these factors on the quality of the biosludge and its feasible use should these deficiencies be eliminated, and we isolated the components and tested them medically and biologically in experiments with animals.

Our objective was to find possible technological measures which could be taken to improve the overall quality. One problem we encountered while carrying out these investigations was that it was necessary to test all modifications in the biological sludge or its isolated components both chemically and analytically in addition to the animal experiments. All this requires time.

The efforts undertaken so far to use quick-assay methods in order to determine for instance the in-vitro digestibility by means of the trypsin test, produced no representative results in comparison to the animal experiments. Differing values were obtained depending on the kind of biomass (see Table 3).

Table 3.

Values found in individual charges of biomass	Digestibility	
	in vitro	in vivo
Excess activated sludge from liquid waste	74,5 %	52,9 %
Methanol-utilizing bacteria	94,9 %	83,5 %
METHANE-UTILIZING BACTERIA	58,5 %	70,9 %

THE USE OF BIOSLUDGE FOR LIVESTOCK FEED

The Assurance of Nonpathogenicity of the Mixed Population

In principle, there are no objections to feeding well-defined mixed bacterial populations if an adequate utilization of the protein component by the animal organism and the absence of pathogenic germs are guaranteed. However, these two requirements are not met if untreated biosludge is involved. The development of a technology of alkaline short-time high-temperature treatment guaranteed the nonapathogenicity required by veterinarians both in the case of feeding the liquid and applying the dry biomass. This process assures complete sterility of the product and minimizes its impact on the content of essential amino acids. After cultivation on a glucose peptone agar at 30-32 C for 72 hours, no growth of microorganisms could be observed.

This technology is also universally applied to biomasses of different origins, for instance when excess sludge obtained in the aerobic treatment of well-defined industrial waste water is involved, should the use of these products for livestock feed be envisaged. But it has not yet been clarified to what extent this procedure can eliminate other undesirable or harmful substances such as antibiotics or disinfectants which are introduced with the liquid wastes into the sewage water when it is aerobically treated. This is a problem which requires further systematic study.

Impact of the Ash Content

The crude ash of a typical biological sludge obtained from treated sewage water from a pig-fattening unit is composed as follows in Table 4.

Table 4

Total crude ash	: 20,7%	/ gross dry substance
Acid-soluble minerals	: 7,3%	
Acid-insoluble minerals	: 4,8%	
Biomass-linked minerals	: 8,6%	

After most of the mineral components had been removed by acid washing, no positive or negative impact on its value regarding nutritional physiology was found. This also confirms the results of Kinzell (1977) who observed no toxic effects when he added the isolated mineral components of biological sludge to the control feeds.

Contrary to the data given by Capar et al. (1978), we did not observe any enrichment of trace elements, especially of a toxic nature, in the activated sludge of liquid waste, regarding the feeds used in connection with the liquid waste itself. The limits recommended by the PAG (Protein Advisory Group) of UNICEF regarding toxic trace elements, were not applied in the samples examined by us.

In our opinion it is not necessary to take technological measures to reduce the ash content by means of acid treatment or ion exchanges if the proportion of biosludge in the total mixture of feedstuffs is low.

Impact of the SO_2 Content

In several large-scale plants concentrated biosludge is dried in a spray drier with direct heating in order to make it storable over long periods of time. The SO_2 content of the biological sludge is due to the uptake of SO_2 from the heating gases of the spray drier and is characteristic of the spray drying of biomass suspensions with a pH value of 7. In animal-feeding experiments no negative effects could be observed.

Technologically the uptake of SO_2 can be prevented almost completely by a weak acidification of the biomass suspension to pH values of 4 to 5 before spray drying (see Table 5).

Biological Value and Digestibility

The low biological value of the biosludge results from an imbalance of essential amino acids, primarily cysteine and

Table 5.

Biomass, spray-dried, flammable gas contact	SO_2-content
Excess activated sludge from liquid waste treatment	0.87 %
The same; acidified before drying pH 4.5	0.04 %
Biomass from methanol-utilizing bacteria	0.00 %
Fodder yeast from molasses	0.00 %

methionine. Tests of N-balance with rats (Kinzell, 1977) concerning the supplementation of biosludge with D, L-methionine resulted in an increase of the biological value from 64.4% to 85.4%, and the apparent digestibility rose by only a small amount, from 63.9% to 68.0%, as was expected. Further animal experiments conducted with different species confirmed this fact.

Under the given conditions, the technological process resulting from this is very simple, and is restricted to the addition of pure amino acids or other suitable feedstuff components having the same effect.

Increasing digestibility presents a problem which has not been solved so far. Our studies have shown - and this accounts for our assumption about the non-toxic nature of the biosludge - that up to 50% of crude protein contained in control feedstuffs can be substituted by an equivalent amount of digestibile crude protein in the form of biosludge. No detrimental influence as regards growth and health of the animals could be observed.

Contrary to assumptions made so far, namely, that the poor digestibility of biosludge is due to both the resistance of the undigestible cell membranes of the microorganisms of mixed population, and the digestible crude protein components linked to them, we determined that low digestibility is in itself a characteristic of protein. Protein isolates obtained from activated sludge showed the same low digestibility as the original substance (see Table 6).

Table 6.

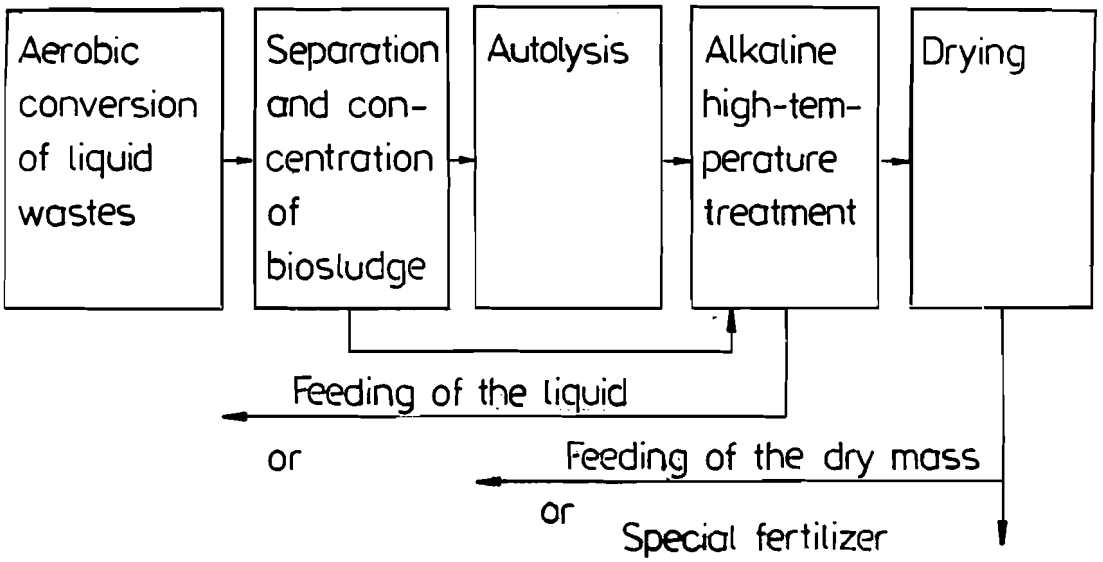
Product	Apparent digestibility
Starting activated sludge	52,9 %
Aqueous alkaline protein isolate	52,0 %
Iso-electrically precipitated protein isolate	52,4 %

The application of chemical hydrolysis in connection with autolytic processes is intended as a technological means of reducing the digestion inhibiting protein structures.

At present, the technological measures needed for the risk-free use of excess sludge in livestock production have still not been decided upon finally.

The questions still remaining undecided can be solved in essence by installing an already existing sewage treatment plant with post-fermentative processes, so that product samples can be produced in larger quantities (see Table 7).

Table 7. Technology of the post-fermentative treatment of the biosludge and possible applications



CONCLUSIONS

Regarding the use of biosludge from the aerobic processing of liquid wastes as a feedstuff component for livestock, the following problems can be regarded as solved:

-- the maintenance of nonpathogenicity;
-- that the relatively high mineral content has no detrimental effects;
-- the maintenance of a high biological value by means of supplementation.

The problems which still remain to be solved are as follows:

-- how to increase the digestibility;
-- how to deal with the possible existence of drugs or disinfectants in the liquid wastes.

Nevertheless, in our opinion, steps such as a revision of the PAG Guidelines should be initiated, and other studies completed, so that the evaluation of the use of biosludge according to international conditions can be made.

In addition to the prior use of biosludge in livestock feed, its application in other possible fields must be investigated, before a final statement can be made as to the safety of its use. One of these other fields of use is in industrial mushroom cultivation.

The Use of Biosludge in Mushroom Cultivation

We studied the possible use of liquid waste biosludge as a protein supplement in industrial mushroom cultivation. Using this method, it is possible to substitute high-value protein sources such as coarse soy-bean meal extracts and malt-seeds, which are normally used as a nitrogen sources, and to surpass them in effectiveness. The time when the biosludge is applied is decisive. In contrast to the abovementioned supplements, it must be applied at the same time as the substrate is inoculated with the mushroom mycelium. A microbial degradation of higher-molecular compounds takes place in the soil, thus enabling the mycelium to receive the nitrogen compounds. This factor render the objection to the use of biosludge in livestock feed because of possible detrimental effects irrelevant. (See Table 8.)

In addition to a significant increase in yield, this technology also helps to eliminate the labor-intensive process of mixing conventional protein supplements after inoculation.

Table 8. Increase in the yield of g mushroom/kg substrate - dry substance with admixture of dry activated sludge (d s) from liquid waste treatment (8.4%)

	without admixture (substrate control)		+ 25 g d s / kg substrate during inoculation	
Day of harvest	Yield		Yield	
8	115	100 %	132	114,7 %
13	190	100 %	283	148,9 %
22	315	100 %	455	144,4 %
27	345	100 %	510	147,8 %

Summary

According to existing local conditions, there are several variants for the use of protein substrates resulting from the classic aerobic treatment of agricultural waste products in the form of excess biosludge.

Regarding material infrastructure, the technologies for the aerobic treatment of wastes from livestock production, and technologies for post-fermentative treatments have to be selected according to the different possible applications of the end products.

It has been shown that the use of heterogeneously composed sewage-water sludge both for feeding livestock and for mushroom cultivation is possible. Its use in mushroom cultivation is unproblematic. However, the starting point for using the process for producing livestock feed is determined by short-time high-temperature alkaline digestion so that its possible use within the framework of zootechnical-toxicological studies can be considered. (See Table 9.)

Table 9.

Genesis of the biosludge	Author	Content (ppm)		
		Hg	Cd	Pb
swine-waste-water	own Results	0.07	0.36	4,2
communal-waste-water	Kinzell 1977	3	5	217
communal-waste-water	Capar 1978	8,6	104	1832
Recommendable maximum value	PAG-Guideline № 15	0,1	no value	5

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THE USE OF ARTIFICIALLY-STRUCTURED PROTEIN PRODUCTS FROM BLOOD PLASMA IN THE MEAT INDUSTRY

I.A. Rogov, N.S. Nadashvili,
and G.G. Mikeladze

One of the most promising trends in the production of meat products with a high food value is the production and use of artificially-structured protein products (ASPP) obtained from the blood plasma of slaughtered animals.

The Moscow Technological Institute of the Dairy and Meat Industry has conducted intensive research into the development of a number of methods for structuring protein systems. These methods can be carried out using ordinary technological equipment, with minimum power consumption, and they ensure the production of ASPP, having a complex of qualities that makes it possible to use them as meat substitutes.

Conclusions drawn from the results of worldwide research on the laws of structure-formation in colloidal systems, the dispersion phase of which is made up of protein micelles, has enabled scientists to establish that methods of producing ASPP may be based on processes of coagulating protein macromolecules. A directional increase in the dispersion medium of the protein-containing solution caused by the hydrogen ion concentration or a polyvalent metal will lead to the compression of the diffusion layer of counter ions, producing a decrease of the Sterne potential of protein micelles, and, as a result, a reduction in the electrostatic component of repulsion energy. When the concentration of counter ions is sufficient for the protein particles to overcome, (due to Brownian motion at a given temperature), the energy barrier emerging during their convergence at some distance between the surfaces of the particles, they coagulate.

At present, the use of ASPP is mainly confined to meat products, the production technology of which includes a stage of thermal treatment. This is linked with the analogy of some properties of a physico-chemical nature present in production

processes and thermal treatment of ASPP, as well as in the process of thermal treatment of meat protein systems.

We are primarily concerned with dehydration (lyophilization) in the initial state of macromolecules of ASPP protein components. This transfer is caused by a directional increase in the concentration of the hydrogen ions H^+ and calcium Ca^{++} in the dispersion medium of the protein-changing system. This leads to a simultaneous change in three factors which account for the amount of adsorbtionally and osmotically bound moisture:

1) compression of the diffusion layer of counter ions;
2) an increase in counter ion concentration in the adsorption layer, and, finally,
3) blocking of ionogenic, amino and carboxyl groups of amino acids forming the initial structure of the protein.

Dehydration is also typical of all fractions of muscular and sarcoplasmic meat proteins, which are heated to a temperature of 70-80°C or more.

It should be especially noted that ASPP and meat protein systems are similar after thermal treatment. This similarity is due to the fact that the overwhelming portion of the moisture is capillary-bound by microstructure cells formed as a result of the coagulation of protein components.

If, prior to thermal treatment, the spatial-continuous coagulation structure of ASPP is of apparently flocculent character, and mainly conditioned by bonds of molecular nature, then, in the course of thermal treatment, the thickness of the solvate layers between separate macromolecules in the protein chains is reduced. Additional covalent and coordination chemical bonds emerge between these walls and lead to a considerable strengthening of ASPP. In the case of equal moisture content, their structural-mechanical properties approach those of thermally-treated meat protein systems.

A comprehensive study of rheological characteristics in nonfat boiled sausages produced in the laboratory, with various doses of ASPP meat substitutes, has shown that optimum dosage lies between 28-32 percent. The number of sausages produced was 103-104 percent in comparison with the control.

A study of boiled sausages with added fat showed that the maximum dosage of ASPP substitution for forcemeat is 35 percent. The optimum dosage lies between 25-30 percent. The sausages produced using this dosage was 104-105 percent compared to the control.

During pilot production of tinned forcemeat the maximum dosage of ASPP meat substitution reached 50 percent. This tinned forcemeat differed very little from the control in organoleptic properties and rheological parameters. However, in this case, the separation of broth was noted during the thermal treatment of tinned forcemeat. The amount of broth was 8-12 percent

of the total tinned weight of the forcemeat. This phenomenon is undesirable, and, due to this factor, the optimum dosage of substitution is considered to be between 30-35 percent.

The possibility of using ASPP in the production of minced semi-finished products has been explored. When substituting for 25-30 percent of meat it was found that the taste of the semi-finished products, which had undergone thermal treatment, did not deteriorate.

Prior to discussing the economic aspects of using ASPP in combined meat technology, it should be noted that this problem is still rather controversial and various factors have to be taken into account. We do not attempt a detailed economic analysis, but rather single out several factors:

1) Firstly, the use of artificially-structured protein products from blood plasma in the production of combined meat products makes it possible to increase output by 1-3 percent.

2) Secondly, the addition of ASPP to combined meat products enables an average saving of between 20-27 percent of meat in the finished product when output is taken into account.

3) Thirdly, the cost of ASPP is two-three times lower than the cost of trimmed meat.

4) Fourthly, technologies for the production of ASPP developed by us make it possible to use blood plasma economically at meat plants where the volume of blood obtained considerably exceeds the demand of sausage production resulting from traditional methods of plasma use.

5) Fifthly, ASPP has a number of properties, which make the simultaneous use of three types of protein-containing raw material (plasma, milk and soybean protein) in the technology of some combined meat products possible.

6) Lastly, the production of all types of ASPP may be carried out at practically any plant where animals are slaughtered and their blood collected, because the standard equipment used at meat and dairy plants can be employed for this purpose.

ISOLATION PROPERTIES AND USAGE OF BLEACHED BLOOD CELL PROTEIN AND ITS PROSPECTIVE USE FOR FEED AND FOOD PRODUCTION

T.D. Mdinaradze, E.S. Davidova,
N.N. Nadashvili, and H.G. Aleksidze

Blood obtained during the slaughter of farm animals, is an important source of bound protein used for food and fodder purposes.

It is known that blood consists of the liquid light-yellow part (plasma) that makes 60% of its mass, with a protein content of about 7%, and of blood cells making 40% of the blood mass, with a protein content of about 40%. Although the use of blood protein for food would have a nutritional advantage, unfortunately, the blood cells owing to their specific smell, color and taste, are mainly used for fodder purposes. About 1% of the quantities produced are used for the production of low-grade blood sausages.

It is known that, for 1 kg protein synthesis, horned cattle consume from 70 to 140 kg fodder and swine up to 50 kg, i.e. for 1 kg protein synthesis they will require at least 150 kg blood cells, taking into consideration the fact that the cell mass is reduced in the process of technical conversion (i.e. their drying) during fodder production. When separating the proteins from blood cells, we can obtain at least 45 kg of protein per 150 kg of blood cells and the food quality of these proteins is similar to that of meat proteins. They are similar in their amino acid content and in their physical and chemical properties.

It should be noted that in the USA there are two methods of obtaining bleached proteins from blood cells, but the isolated protein has low emulsifying ability, and a large number of reagents (such as chloroform, ascorbic acid, acetone, hydrochloric acid, spirit-ether solution and caustic soda) are used for eliminating this defect. This makes the process complex

and requires expensive equipment, taking into consideration also that spray-drying of the product is a further stage of the process; this makes the product expensive.

A simpler method was investigated for obtaining bleached proteins from blood. The blood cells of slaughtered animals, obtained during blood separation, were used as the initial raw material. Blood cells were haemolyzed and transferred to acetone solution and treated with an acid. The mixture was filtered by means of vacuum on the Nutch-filter. The separated bleached blood-cell proteins were additionally washed with acetone until completely bleached and then dried at 40°C. The whole process lasted about 5-6 hours. Drying and acetone removal was carried out at 40°C or under conditions of intensive air movement in an exhaust-hood without heat supply for 4.5-5.5 hours. The completion of drying was determined by testing for the disappearance of acetone traces in the proteins by the nitroprusside method. After each cycle the used acetone was distilled for further use. The acetone loss by distillation was about 8-10%. The separated protein preparation had a white color and no smell; its pH solution is from 2.5 to 3.3.

The chemical composition of the protein preparation is shown in Table 1.

Table 1. The chemical composition of the protein preparation

Components	Contents
Moisture	4.0-4.5
Protein	89-93
Ash	2-3
Lipids	1-2
Nucleic acid components	0.024
Mineral substances:	
Na	0.2
K	0.048
Ca	0.026
p	0.2

The amino acid content of bleached blood cell proteins was determined by hydrolyzing in 6 NHCl over a period of 24 hours at 110°C and analyzing the amino acids in the hydrolysate with an amino acid autoanalyzer ("Hitachi" Japan).

According to the content of the majority of the amino acids, our results are consistent with the data for amino acid content of haemoglobin

Table 2. Essential amino acid content of bleached blood proteins

Amino Acids	g. content per 100g proteins		
	BBCP	in globin	FAO standard
lysine	19.90	8.51	4.2
histidine	8.90	8.7	
arginine	4.0	3.65	
Asp. acid	9.6	10.6	
Glut. acid	8.90	8.5	
serine	4.60	5.8	
proline	3.7	3.9	
threonine	5.20	4.36	2.8
phenylalanine	4.2	7.7	2.8
tyrosine	2.10	3.03	
glycine	4.02	5.6	
alanine	7.40	7.4	
cystine	0.0	0.45	
methionine	1.60	1.7	2.2
valine	9.0	9.1	4.2
isoleucine	1.20	-	4.2
leucine	10.8	15.4	4.8

The above data shows that the bleached blood proteins contain all the essential amino acids and are a good source of the essential amino acids - lysine and leucine. The level of such essential amino acids as threonine, valine and phenylalanine also exceeds the FAO standard, thus showing the high food value of these products. However, the contents of the sulphur-containing amino acids - cystine and methionine is somewhat limited.

In order to apply bleached blood cell proteins in the food industry, we studied their rheological properties for example water-binding (WAC%), fat-binding (FAC%), and foam producing (FOP%) properties.

It was shown that 1g of bleached proteins could hold 8.5g of water and 0.5g of fat. Data obtained from studies on the foam-forming properties of proteins indicate a decrease in the foam-forming level with an increase in the bleached protein concentration. This property reaches its maximum at 2% bleached protein solution.

The bleached protein solubility appears to be highly dependent on the medium pH (Figure 1). These proteins are highly soluble at the pH range from 1.5 to 5.0 and from 9.0 to 11.0. The solubility of bleached proteins decreases at the pH interval from 6.0 to 7.0 and from 6.5 to 6.7 it is significantly reduced.

In preliminary studies on the conditioned reflexes of rats, the physiological effectiveness after feeding with BBCP for a month, was evaluated (the feeding of 150mg/kg animal weight was carried out according to the lysine content in BBCP). The results of studies of the effects of BBCP feeding on weight gain showed that it promotes a rapid increases in the average weight of rats (Figure 2). The difference in weight increases between control and experimental rats was 29% and 44% respectively. Moreover, feeding BBCP to animals improves their resistance 1.5 times (Figure 3).

Based on the above-mentioned facts, we may conclude that the protein preparation, besides its high food value, also improves automatic reflexes. We have therefore started to carry out experiments on the use of BBCP for food and fodder purposes. Interest in this project was also prompted by economic factors. For example, by dissolving 2 kg BBCP in 8 kg water, we obtained a product which was similar to forcemeat in consistency, and had a 18-19% protein content.

A balanced animal feed was prepared with the BBCP and added fats, sugar and minerals. The feed was tested by researchers at the Georgian Veterinary Institute on twenty day old sucking pigs. Tests proved that these substitutes have a feed value similar to that of natural cow's milk.

Based on our experiments, we conclude that the BBCP obtained has a high nutritional value and can be used in the production of food stuffs.

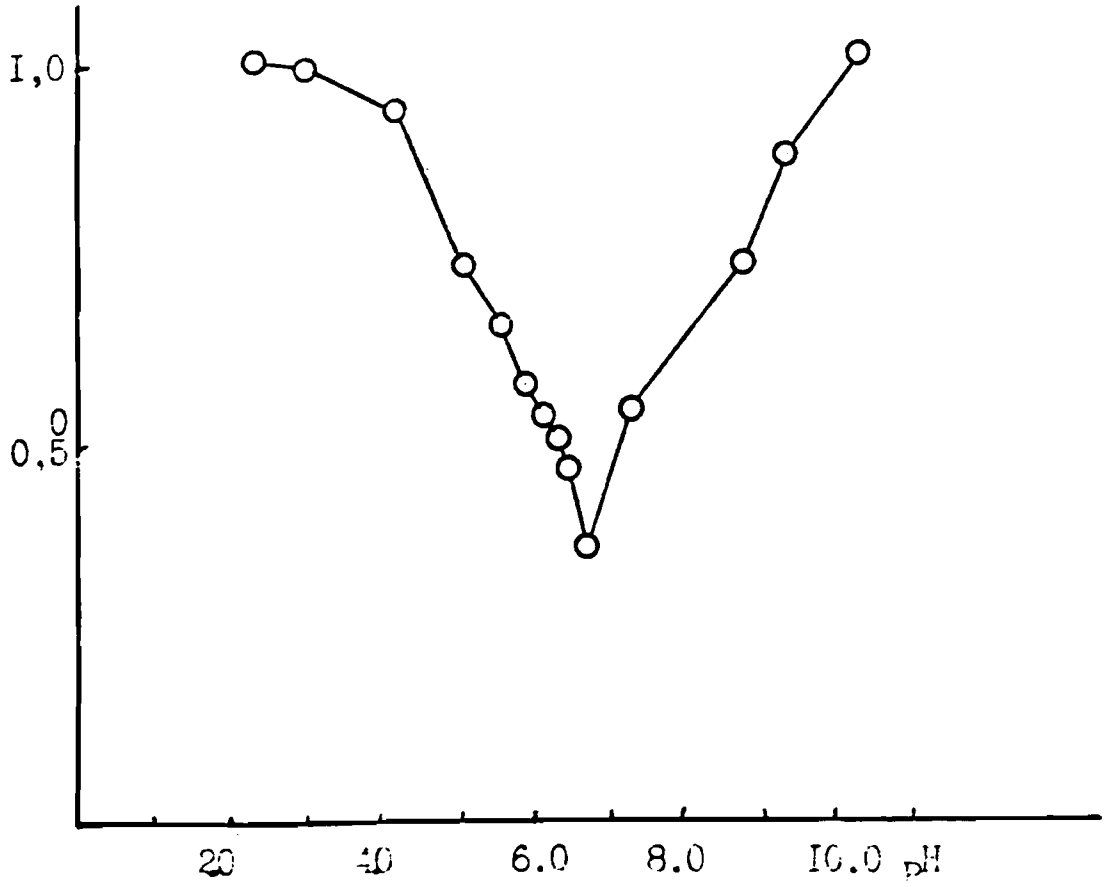

Figure 1. The dependence of BBCP solubility on pH

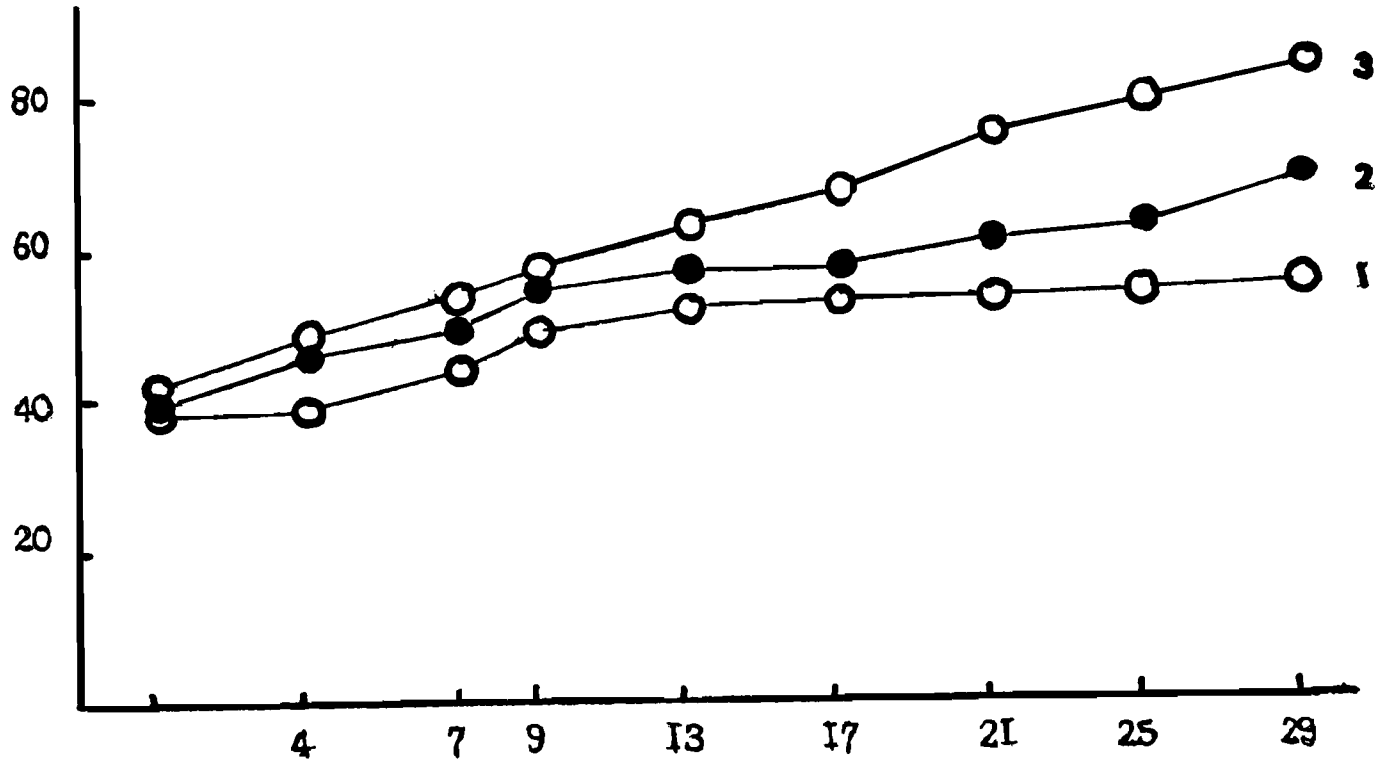

Figure 2. Weight changes in animals during BBCP feeding:
1 - control;
2 - BBCP feeding of animals (700 mg/kg weight);
3 - BBCP feeding of animals (150 mg/kg weight);

The abcissa indicates the days, the ordinate indicates the weight of the animals.

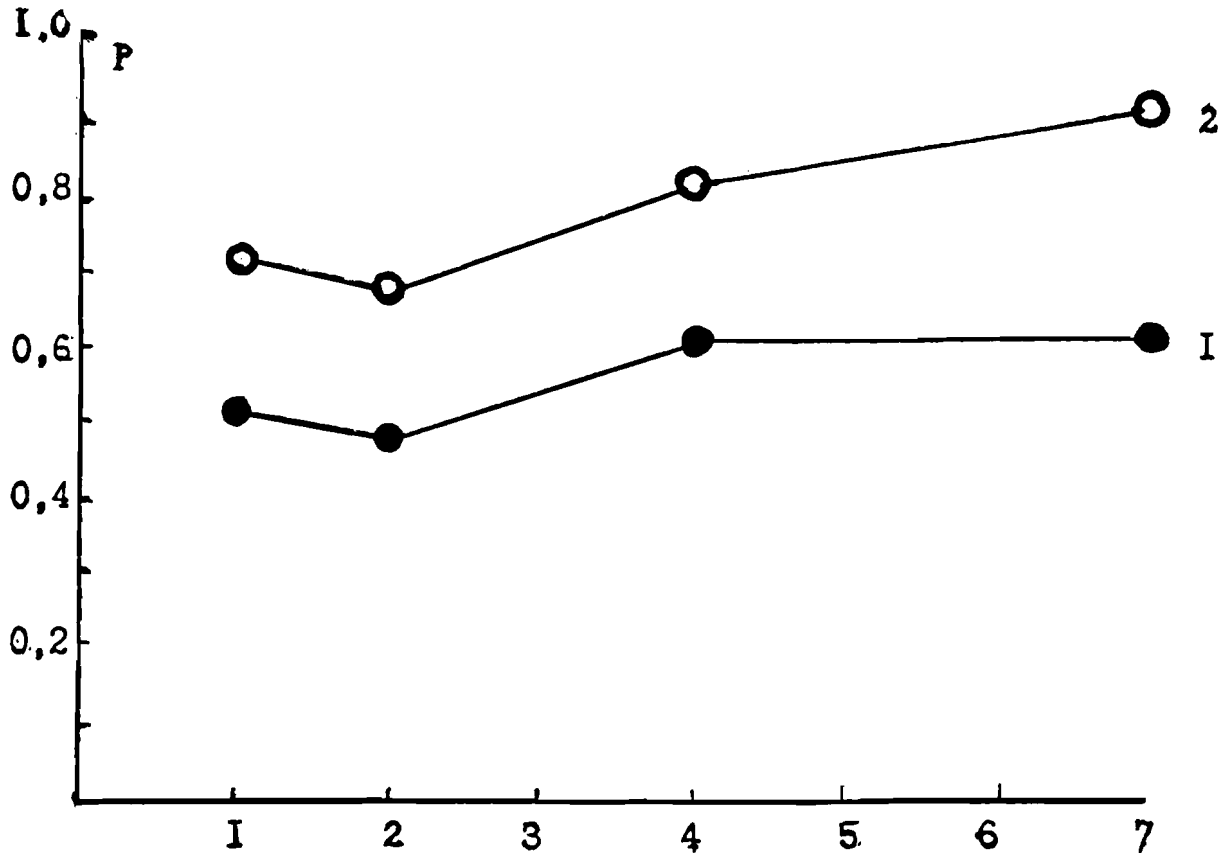

Figure 3. The effect of feeding BBCP on the conditional reflex memory of animals:

1 - control;
2 - BBCP feeding of animals;

P - the ordinate (reaction probability);
- the abscissa (the days)

FOOD AND FODDER ENRICHMENTS FROM GRAPE WINE YEAST PRECIPITATES

L.A. Mudzhiri

Chemical and biological research has shown that the secondary products and wastes of the wine industry contain enormous amounts of biologically active substances (Mikeladze 1980; Mudzhiri 1976; Mudzhiri 1980; Mudzhiri 1981; Razuvaev 1975) which can be used in agriculture for the enrichment of fodder and as fertilizer, in medicine for producing important drugs, in the food industry for making concentrates, extracts, food additives and tonic drinks, and also for manifold purposes in the chemical, biological and other industries.

Yeast precipitates of wine fermentations provide many possibilities for the production of nutritive concentrates, as they are known to contain proteins, free amino acids, reducing sugars, fatty acids, vitamins, and other biologically active compounds. The total amount of nitrogenous substances in yeast precipitates from wines is about 40%, 25-30% being made up of proteins. The content of proteins and other nitrogen-containing substances depends on the yeast culture used, on the type of grapes, the technological process and other factors. In addition to proteins and amino acids, peptides make a very interesting class of nitrogenous biologically active compounds. Protein chemistry which has been intensively developed in recent years has considerably increased knowledge of the chemistry of peptides. The techniques employed in protein chemistry, e.g. electrophoresis, chromatography, ultracentrifugation, automated determination of the content and sequence of amino acids, X-ray analysis, ultraviolet and infrared spectroscopy, mass spectrometry, gel filtration, and other methods of purification, isolation and structure determination, have been extended and used successfully for many newly discovered natural peptides.

Peptides play multiple roles in the life processes of animals and microorganisms. Their most important functions, in our view, are that they act as hormones which enhance anabolic processes, e.g. the level of biosynthesis of proteinaceous bodies. Some aspects of the functional activity of peptides, e.g. their structural role in RNA and DNA, participation in enzymatic catalysis, in the biosynthesis of nucleic acids and proteins, and, last but not least, their metabolic role in the de novo formation of a number of proteins (Fillippovich 1963) are also significant.

The aim of our research was a study of the peptides and proteins of the yeast precipitates of wine and the development of effective procedures for isolating these compounds.

It is known that peptides can be firmly attached to proteins and their removal can only be achieved by special methods.

Peptides are often isolated from biological materials together with amino acids, which means that their chromatographic removal may present difficulties. The first step in our work was to prove that yeast precipitates from wine contain a peptide fraction; after that we tested and compared various isolation procedures. We isolated peptide and protein fractions by different methods, i.e. the Winnick method (Winnick et al. 1955), the Sisakyan and Veiniva method (Sisakyan et al. 1962; Veinova et al. 1966), the Porath method (Porath 1957), the Mitchell and Simmonds method (Mitchell and Simmonds 1962), and the Singe method (Singe 1964). The peptide and protein fractions isolated from wine precipitates by the above methods were then thoroughly studied. The quantitative amount of amino acids was compared in hydrolyzed and non-hydrolyzed preparations. Increases in the amount of amino acids after hydrolysis were measured as a factor showing the presence and character of the protein and peptide materials. The protein and peptide material was fractionated in ion exchange columns packed with Sephadex. The resulting peptides and proteins were identified spectrophotometrically, by ninhydrin reactions and according to Lowry.

It was established that the yeast precipitates of wine contain abundant and varied peptide and protein material. The best results were achieved with the help of Mitchell and Simmonds' method: extraction by 75% methanol at low temperature followed by fractionation yields short peptides, lipid derivatives of peptides, long peptides, and proteins.

In order to increase the yield of peptides and proteins, we tested some enzyme preparations that can lyse yeast cell walls. We wanted to increase the amount of nutritive value compounds in the hydrolyzates. At our disposal we had some lysing enzyme preparations developed at the Vilnius Research Institute of Applied Enzymology. The G-3x preparation with a lysing activity of 20,000 units/g (Devdariani et al. 1981) proved to be the best. The degree of hydrolysis of yeast cell walls was judged by the quantity of soluble proteins, amine nitrogen, reducing sugars, free amino acids and fatty acids in the samples and the

controls. Soluble proteins were tested according to Lowry as modified by Miller, Schacterle, and Pollach (Schachterle and Pollach 1973); amino nitrogen by the Truskavetsky method (1973); reducing sugars calorimetrically as described by Noelting and Berufeld (1948); free amino acids were identified in an LKB Biocal 3201 amino acid analyser. Analysis of fatty acids was performed by gas chromatography (Stevens and Glenn 1965).

To see how the yield of the enzymatic hydrolysis of wine yeast depends on the amount of the enzyme preparation, we experimented with different concentrations of G-3x. We prepared an aqueous suspension of yeast. Hydrolysis was carried out for 4 hours with periodical stirring. The pH of the hydrolyzed mixture was checked every hour and, when necessary, adjusted to 8.7. After hydrolysis, the volume of the hydrolyzed mixture was measured and it was centrifuged at 3,000 r.p.m. for twenty minutes. The centrifugal was collected and lyophilyzed. This investigation showed that addition of the G-3x enzyme preparation always considerably increases the quantity of soluble proteins, amino acids and reducing sugars in the hydrolysates. To find the optimal conditions for enzymatic hydrolysis for large scale production, various alternatives were tested including preliminary distilllation of alcohol from yeast.

These experiments demonstrated that treatment of the vinasse by G-3x preparation increases the yield of hydrolyzate containing nutritive value compounds almost five-fold.

In the factory, the treatment of wine yeast slop involves distillation of ethyl alcohol and the precipitation of calcium tartrate. Therefore we developed a special procedure for the production of peptide and protein isolates using the above enzyme preparation. A dense sour cream wine precipitate was subjected to distillation to obtain ethyl alcohol. The slop was supplemented with a 30% soda solution, and an aqueous suspension of G-3x of a lysing activity of 20,000 units/g was added with periodical stirring. Then the mixture was filtered and the resulting liquid neutralized to precipitate calcium tartrate, by the addition of calcium chloride at 50°C. After precipitation of calcium tartrate, the hydrolyzate was filtered, concentrated and extracted by ethyl ether or chloroform. The extract was evaporated to dryness under reduced pressure (the first fraction). Then two parts of methanol were added to the hydrolysate to obtain a precipitate. The precipitate was separated from the solution and dried in a vacuum (the second fraction). This precipitate was treated three times with hot water for five minutes each time. The washed precipitate was separated from the solution and dried in a vacuum or lyophlyzed (the third fraction). The residue remaining after the solvent extractions was dried (the fourth fraction).

All four fractions were subjected to chemical and biological tests. The first fraction was found to contain lipid derivatives of peptides and other low molecular weight biologically active compounds; the second fraction contained amino acids and short peptides, the third fraction contained long peptides and the fourth fraction, proteins.

To determine the amino acid composition of the peptides and proteins, they were subjected to hydrolysis in 20% HCL at 105°C for 24 hours. Analysis of the hydrolysates showed that the peptide fractions contained from 5 to 12 amino acids, with lysine, glycine, arginine, aspartic acid, threonine, proline, valine and leucine being prevalent. The amino acid composition of the proteins from the yeast precipitate is the following: lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, - phenylalanine and tryptophan.

The above fractions were tested in vitro and in vivo. The in vitro tests were performed with four lines of continuous cell cultures by adding the protein acid peptide fractions. All the samples were incubated at 37°C for 24 hours. After incubation, the total nucleic acid content in each sample was measured. The increment in the samples after incubation was determined by comparison with the initial content. In vivo tests were carried out by adding 4,000 mg/kg of the peptide and protein concentrates to animal food. In 10 days a 7% increase in weight was registered compared to the control.

The above chemical and biological tests allowed the conclusion to be made that the peptide and protein isolates prepared as above, possess a considerable growth-stimulating activity. Toxicity tests showed that the isolates can be regarded as harmless.

Thus, the peptide and protein fractions isolated from wine yeast precipitates can be used in the agriculture and fodder industry for enrichment of fodder, and the protein fraction can be used in the food industry for the enrichment of food stuffs.

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THE SMALL-SCALE PRODUCTION OF EDIBLE PROTEIN FROM BY-PRODUCT LEAVES

N.W. Pirie

Many research institutes, in several countries, are studying the extraction of protein from leaves because:

1. The leaf if the site of protein synthesis and there are losses when protein is translocated to seeds or tubers.

2. When suitable leaf crops are harvested, there is photosynthetically active green cover on the ground throughout the growing period. Yields are therefore greater than those from crops which occupy the ground while merely ripening. When perennial crops are used the ground is protected from erosion.

3. When a crop is used as ruminant fodder, 10 to 25% of the protein in it is converted into human food, whereas 50 to 65% of the protein can be extracted and the unextracted protein is still available for ruminants.

4. The processes of extracting and separating leaf protein (LP) disintegrates the leaf and removes toxic or ill-flavored components. Species normally rejected as sources of human or animal food can therefore be used.

5. For satisfactory protein extraction, a forage is harvested when young. The fibre is therefore less lignified than when a crop of hay is taken. Furthermore, the crop is not at risk from pests and diseases for so long.

6. The process of extraction removes most of the water from the fibre. If it is ensiled there if therefore no drip; if it is conserved by drying, less fuel is used.

Points 1, 2 and 3 were the stimulus for the initial research, but the importance of point 6 was repeatedly stressed. Industrialists, and those responsible for national and international research policy remained unconvinced. They doubted the palatability of LP and they assumed, in spite of the experience of all those working on the process, that extraction would be very expensive. Passage of time, and the recent increase in the cost of fuel, have increased awareness of the importance of point 6, consequently, crops are now being fractionated to make feed for ruminant and nonruminant animals in France, Hungary, Spain and the USA. The level of interest in fodder fractionation shown in Denmark, Egypt, India, New Zealand, Pakistan and the Philippines, as a means for making human and animal food, suggests that regular production in these countries is imminent. This partial diversion of interest from human to animal feeding seems illadvised to those who are concerned with human nutrition, especially in the wet tropics where points 2, 3 and 5 are important and malnutrition is common. The diversion has however the merit that it will supply figures for processing costs and so demolish one argument against the mechanical fractionation of leafy crops.

SOURCES OF LEAF

When crops are grown primarily as sources of LP, with ruminant fodder as a by-product, the yield of dry 100% protein can be 2 t ha^{-1} y^{-1} in temperate climates, and twice that in regions where there is no winter cessation of growth (Pirie, 1978). Yields so much large than those attainable by any other method for producing a protein concentrate are only achieved with lavish use of fertilizer: it may be more practical to aim at yields only 2/3 as great. When by-product leaves which would otherwise be wasted, can be used, the case for protein extraction becomes even stronger although, because the leaf supply is intermittent, annual yields cannot be as great as from crops grown in succession specially for extraction. Protein cannot be easily extracted from all types of leaf. Fibrous or dry leaves, and leaves that give acid or phenolic extracts do not extract well: extracts from glutinous or slimy leaves are difficult to handle. In spite of these limitations, more than 100 species are known from which extraction is satisfactory. Unfortunately, some of the more abundant by-products have defects that make them useless or improbable as sources of LP. For example, cereal straws are too dry by the time grain is harvested. Sugar cane tops are moister but very fibrous, and they seldom contain more than 1.2%N on the dry matter (DM). That, however suggests that they contain about 500 kg of protein ha^{-1}. Balasundaram et al. (1974a) extracted 108 kg of LP ha^{-1} from them. Cane tops are usually burnt off to make hand harvesting easier and pleasanter. With mechanical harvesting, it would be easy to separate the moist green tops from the drier trash.

The area devoted to cassava (tapioca, manioc, or yuca, Manihot esculenta or utilissima) is so large, and there are such extensive plans for increased production for food and industrial alcohol, that thorough study of its leaf would be worthwhile. Varieties differ; this may explain why Byers (1961), Singh (1964) and Balasundaram et al. (1974a) extracted little protein from it, whereas Balasundaram et al. (1974b) and Fafunso & Oke (1977) were more successful. Unfortunately, these papers do not state that the leaves were a genuine by-product taken at the normal time for harvesting the tubers. On the other hand, efficient extraction equipment was not used in these experiments and cassava leaves, when mature, are dry and tough. Other by-product leaves from which LP is not likely to be economically extracted, are those cultivated for the sake of essential oils or perfumes; they are usually subjected to drying or steam distillation, and these processes coagulate protein in situ.

Sugar beet (Beta vulgaris) and potato (Solanum) leaves are the two most abundant by-products in the temperate zone. Yields from the former, which is largely wasted in Britain though not in Germany or Poland, depend on the weather and the date of harvest; the largest yield during casual trials at Rothamsted was 500 kg LP ha^{-1}. However, even if the yield in commercial practice were only half our experimental yield, the 193,000 ha on which sugar beet is grown in Britain would produce 48,000 t of extracted protein as well as a fibre residue that would be more attractive cattle fodder than the untreated tops. Much larger amounts of LP could be made in Europe: Plaz & Chartier (1980) estimate that sugar beet tops in the EEC contain 13 Mt DM.

The outstanding photosynthetic efficiency of sugar beet is due in part to the vertical disposition of its leaves (cf. Monteith, 1977). Towards the end of the growing season, the leaves bend over so that there is more mutual shading and a decline in efficiency. A few experiments suggest that part of the leaf can be harvested early in the year with little diminution in sugar yield - presumably because the new growth keeps more nearly vertical. The idea that some leaf can be harvested from a crop without affecting the yield of the primary product deserves fuller investigation.

As a safeguard against blight, and to facilitate tuber-lifting later, potato haulm is usually destroyed mechanically or with a herbicide at the beginning of September. The main reason for the neglect in Britain of potato haulm as an animal feeding stuff, is fear of poisoning by solanin and other glyco-alkaloids. These are, to a large extent, removed from both LP and the fibrous residue during processing. LP has been made regularly from potato haulm at Rothamsted since 1952, it has also been made in India, Pakistan and Poland. In nutritive value for rats it resembles LP from other sources (Henry & Ford, 1965; Hanczakowski, 1974; Hanczakowski & Makuch, 1980).

The yield of LP depends on the potato variety and the date on which the haulm is taken (Carruthers & Pirie, 1975; Hanczakowski & Makuch, 1980). Some early varities yield 600 kg

ha^{-1}, maincrop can yield 300 in late August, but yield diminishes to 100 or even less by mid-September. Once the idea has been accepted that something valuable could be extracted from potato haulm, prudent farmers would probably remove haulm a little earlier than usual so as to prevent blight. But tuber weight is still increasing at the beginning of September, and it would therefore seldom be worthwhile to take the haulm early simply to increase the yield of LP. In Britain, early potatoes occupy 30,000 ha and main crop 230,000 ha. It is reasonable to conclude that about 50,000 t of LP could be extracted from the haulm.

Mobile viners now drop pea (Pisum sativum) haulm in the field. The whole crop used to be carted to the viner, and the haulm was sometimes used. From haulm grown on experimental plots, 600 kg LP ha^{-1} can be extracted: that is more protein than is in peas. Haulm collected from a factory 30 km from Rothamsted did not extract as well as fresh haulm - perhaps because there was an interval of about 3 h before the bruised and battered haulm was pulped. The percentage of haulm N that was recovered as protein N varied from 22-47% (Byers & Sturrock, 1965). If we reverted to the old method of vining in a factory, protein could be extracted from the haulm without delay. However, peas may not ultimately be a useful source of LP because there is little leaf on some new varities.

When maize is allowed to ripen completely, the leaves are too dry and depleted of protein for satisfactory extraction. From some varieties, harvested at the end of August, only 20% of the protein was extractable (Byers & Sturrock, 1965). When harvested earlier, i.e. at the sweet-corn stage, nearly half the protein was extractable and the yield of extracted protein reached 480 kg ha^{-1}. The area devoted to sweet-corn in Britain is too small for it to be an important source of LP. Tomatoes, on the other hand, occupy a large area in many countries: protein has been extracted from their vines after harvesting the fruit (Kramer & Kwee, 1977).

Open air vegetables occupy 0.17 M ha in England and Wales - the brassicas alone occupy 60,000 ha. Raymond (1977) quotes 4.6 M t as an estimate of the total fresh weight of discarded material: Palz & Chartier (1980) estimate that 3.7 M t DM is available in the EEC. That probably contains 0.7 M t of protein and half of it would be extractable. Material discarded in the field would be fresh and worth using as a source of LP. Much of it is already being collected and some market gardeners have to pay for its disposal. Discards at the retail level will probably be too damaged and withered to be worth extracting.

Tekale & Joshi (1976) point out that the cultivation of vegetables gives Indian farmers a better income than other types of farming, and that recent improvements in vegetable varieties are as sensational as those with the widely publicised new cereal varieties. A survey by FAO (1971) found that vegetable consumption in India was almost the smallest in the world. It is therefore likely that market gardening will soon increase

greatly, and it is fortunate that several by-product leaves have already been studied there. Yields of LP from the brassicas were 90-160 kg ha^{-1} (Matai et al., 1973; Deshmukh et al., 1974; Tekale & Joshi, 1976). LP made from cauliflower leaves, taken as a by-product, was a useful supplement to wheat in a rat diet and contained 0.6 mg β-carotene g^{-1} (Goel et al, 1977). Other useful by-product leaves from market gardens were chicory (Cichorium intybus) (Mahadeviah & Singh, 1968), beetroot (Tekale & Joshi, 1976; Bagchi & Chanda, 1980) and sweet potato (Ipomoea) (Byers, 1961; Balasundaram et al., 1974a, b; Deshmukh et al., 1974). Protein has also been extracted from sweet potato leaves in the USA (Walter et al., 1978). Hussain et al. (1968) estimate that 60,000 t of LP could be extracted, half from radish (Raphanus) leaves, in Pakistan. In India, Tekale & Joshi (1976) and Bagchi & Matai (1978) got yields up to 134 and 286 kg ha^{-1} respectively from radish leaves. The potentiality of groundnut leaves (Arachis hypogaea) depends on the rainfall in the period before the seed is harvested. In Ghana they extracted reasonably well (Byers, 1961); in India and Nigeria poorly (quoted in Pirie, 1971).

Plants are grown on such a large scale as sources of fibre that these leaves deserve careful study. LP has been extracted from cotton (Gossypium hirsutum), but it would be difficult to collect leaves after the usual procedures of chemical defoliation. The leafy upper part of ramie (Boehmeria nivea) is cut off and left in the field before the stem is harvested (Byrom, 1956), and the various tall species from which jute and similar fibres are made, usually have to be scutched in the field before retting. Some of these leaves are inconveniently mucilaginous: this may be a varietal matter and so could be avoided. Some of the other fibre-plants are less probably sources of LP. Sisal (Agave sisalana) has an acid leaf, that defect could be partly counteracted by scutching in dilute sodium carbonate. Abaca (Musa textilis), like banana (Musa sapientum), contains phenolic material that will probably interfere with extraction. Although these difficulties may be overcome, it would be unwise to assume that every protein-rich leaf is a potential source of LP.

Much of the protein in deciduous tree leaves autolyses in autumn to products that return to the roots; the leaves that are finally shed are too dry to extract satisfactorily. There are chemical treatments that cause leaf fall at other times of year. This method of collection would be worth study; otherwise collection difficulties make it unlikely that fully grown trees, felled in the course of conventional forestry, will be a useful source of LP. The straight unbranched habit of coppiced trees simplifies mechanical leaf stripping. Coppiced trees are already extensively grown as sources of firewood and paper pulp, and several countries plan the cultivation of "energy plantations" for industrial fuel. When species are being selected, some attention should be given to the extractability of the protein in the by-product leaves. Unsystematic studies at Rothamsted suggest that protein tends not to extract as readily from the leaves of trees as from other types of plant. Elder (Sambucus) is the best that we have found: Carlsson et al, (1980)

extracted protein from a eucalyptus, Byers (1961) from Leucaena and Balasundaram et al. (1974b) from Gliricidia. There are many more species that deserve study.

Work such as this is important because a food-producing tree crop would be the ideal replacement for tropical rain forest. It is now obvious that ecological disaster follows attempts to cultivate annual plants in regions where there is frequent intense rain. Unless the soil is protected by the roots of perennial plants, erosion is a serious risk. The trees usually thought of as replacements for natural rain forest produce an exportable commodity such as rubber or palm oil, and it would be advantageous if by-products from some tree crops were used to produce protein for local consumption.

The untended, mixed growth of weeds on land is not a potential source of LP although some individual species from the mixture may ultimately be cultivated as sources. The wild growth is unsuitable because it is unmanured and grows on rough sites from which collection would be difficult - otherwise it would not have weeds on it. The situation is different with water weeds. Obviously they do not suffer from drought, and they are often abundantly manured. The excessive growth of water weeds increases the waste of water by evaporation in hot countries, interferes with flow in irrigation ditches, interferes with navigation, and is a health hazard when it harbours disease vectors such as snails. Much effort is therefore expended on attempts at control. Mechanical destruction, herbicides and "biological control" leave weed remains in situ. These methods may control growth, but they do not affect eutrophication. The killed plants rot in the water and most of the elements they contain, and the excreta of an unharvested agent of "biological control", are likely to return there. When herbicides are used, they and their breakdown products may remain in the water and so make it unsuitable, or less suitable, for irrigation. If water weeds were used, and so removed from the water they infest, that water would not only be freed from infestation, but would be depleted of the elements causing eutrophication. This would economically convert a problem into an asset.

Water hyacinth (Eichhornia crassipes) is the most abundant, troublesome and decorative of the weeds. It is said to cover 200,000 ha in India, and similar areas elsewhere. The total area is probably more than 1 M ha with an annual growth of 10-30 t DM ha^{-1}. The DM of the whole floating plant contains about 2.5% N, the leaves contain up to 5%. An impressive amount of protein is therefore potentially available. Unfortunately it does not extract readily unless alkali is added (Ghosh, 1967; Taylor et al., 1971; Matai, 1976); extraction is being commercialized in the Philippines (Monsod, 1976). Pieterse (1974) reviewed the literature on the biology and use of water hyacinth in a perhaps unreasonably pessimistic manner; in a later (1978) review, with 666 references, he was more optimistic. It has been obvious for 30 years that hyacinth is a potential source of fibre. Paper is now being made from it in India: it is to be hoped that it will not take 30 more years to extract LP from it as well!

Two other floating weeds, nile cabbage (Pistia stratiotes) and the fern (Salvinia auriculata, extract as badly as water hyacinth at their natural pH (about 6)(Byers, 1961; Matai et al., al., 1971; Matai, 1976; Rothamsted unpublished). The effect of adding alkali to them has not apparently been tried.

It would be easy and economical to collect these floating weeds with equipment mounted on a barge, process the extract on it, and discharge the soluble material. The compact extracted fibre and LP would have to be transferred to land less often than would be necessary with bulky fresh weed. That method of working would obviously not control eutrophication as effectively as processing on land.

Protein appears to extract more easily from rooted than from floating water weeds: there is no obvious physiological basis for this distinction. From mixed weeds collected in Hertfordshire, 47% of the protein was extractable (Pirie, 1959); from a water lily (Nymphaea lotus) in Ghana, 40% (Byers, 1961); and from one in Alabama (Nymphaea odorata) 61% (Boyd, 1968). Boyd (1968, 1971) lists several other species that extract well, Justicia americana yielded 300 kg of LP ha^{-1} when harvested in May or June. Nothing seems to be known about the readiness with which these plants regrow after harvest. Unfortunately reeds (Typha and Phragmites), which produce very heavy crops in suitable regions (Dykyjova, 1971), do not extract well. So much effort is now expended on not very successful attempts to control water weeds, both floating and rooted, that sustained work on finding uses for them is likely to be profitable. The subject is now getting more attention (US National Academy of Sciences, 1976; Pirie, 1980).

PREPARATIVE TECHNIQUE

Protein cannot be extracted from leaves by simple pressure. The leaves must first be rubbed or disintegrated, and it is more important to maintain pressure on the pulp for several seconds, so as to allow juice to run out of the fibre, than to apply intense pressure. From adequately pulped leaves, 80% of the juice that can be expressed with intense pressure, is expressed at 2 to 3 kgf cm^{-2} (200 to 300 kPa). Pressure is not sufficiently prolonged with rollers, and conventional screw expellers do not disintegrate the crop sufficiently before applying pressure. Hitherto, most research on LP has been concerned with the quality of the product and the yields that could be expected from different types of leaf. The cost of the equipment and the amount of energy used in the extraction were secondary considerations. Now that the merits of LP are recognized, the economics and practicalities of extraction deserve much more attention.

Somewhat different problems arise according to whether production is envisaged on the domestic, farm or industrial scale. These scales may be roughly classified as the daily processing of 10 kg, 100 kg and 100 t of crop (wet weight) and these

quantities would produce, respectively, about 250 g, 2.5 kg and 2.5 t (dry weight) of extracted protein. At present, there is no satisfactory equipment for use on the domestic scale. Juice can be made with a domestic mincer followed by hand squeezing in a cloth - but the process is tedious. The best arrangement will probably be a pestle lifted by prongs on a pedal-driven wheel and allowed to drop on to leaves in a mortar with a strong, perforated bottom. More leaf would be added as the pulp came through, and juice would be expressed in a simple hand press.

Equipment for industrial production is being studied in several institutes - notably by Professor Bruhn and his colleagues (Nelson et al., 1980) in the Department of Agricultural Engineering of the University of Wisconsin (Madison, USA). They force the crop out through perforations in a cylindrical die by means of an internal roller, i.e. by an action similar to that of a pelleting press. Juice is then expressed in a separate unit.

For farm scale production we (Butler & Pirie, 1981) use a modified screw expeller. Juice is liberated by rubbing the crop thoroughly with a set of angled paddles which push the pulp into the section of the unit in which it is pressed. That section consists of a perforated cylinder within which an auger rotates. This is mounted on a cone with its wide end at the outlet of the cylinder. A thin shell of pressed fibre emerges there, and the juice comes through the holes (3 mm) in the cylinder. With a cylinder 160 mm in diameter, the unit takes 300 W when fed at 1 to 2 kg min^{-1}. Power consumption and rate of working obviously depend on the texture of the crop. The unit runs at 10 to 15 rpm and so could, if need be, be powered by an animal. Equipment for production on this scale is at present in most need of skilled work on design so that a unit that is cheap, economical and robust can be supplied to those engaged in research, or for routine production of LP from local material for local use.

Leaf juice is heated to 70°+ to coagulate the protein, and this is collected on a filter, washed if necessary, and pressed to a hard, moist cake. It can be preserved in all the usual ways. The DM contains 60 to 70% protein and 20 to 30% lipid - about half of it doubly or trebly unsaturated. Like other mixtures of protein and lipid, LP is damaged by drying unless great care is taken. These techniques are described in more detail elsewhere (Morrison & Pirie, 1961; Pirie, 1978).

USE OF THE PRODUCTS

Fodder fractionation by the methods outlined here produces LP, fibrous material from which much of the protein has been extracted, and an aqueous solution containing amides, salts, sugars etc. All three are valuable and have to be used if the process is to be viable on a farm. It will be less important to use all the products if the starting material is a water weed or some similar waste. Most attention is paid in this paper to LP because there is too little protein in the diets eaten in many parts of the world where leafy material is available, or

could be cultivated, abundantly. This is not the place to discuss human protein requirement. All that need be said is that, after a phase in which energy deficiency was said to be the only serious problem in world nutrition, many experts are now returning to the older opinion that protein deficiency is widespread. Obviously, unless energy needs are being satisfied, protein concentrates will be wasted. But it is easier to produce crops that contain too little protein to form the basis of an adequate diet, e.g. bananas, cassava and rice, than to produce protein concentrates with which to fortify them. If this were not widely agreed, less effort would be put into animal husbandry, fishing, and the cultivation of legume seeds. Extensive trials in India, Nigeria and Pakistan show that LP is both useful and acceptable as human food. These trials have been surveyed elsewhere (Pirie, 1978). LP for them was usually made from plant species used in conventional agriculture. Trials should now be extended to include LP from by-product leaves and wastes. The evidence published so far suggests that differences in amino acid composition between LP from different sources are small. LP from some species does, however, retain a strong and often unpleasnat flavor. Flavor can be judged with small samples made in the laboratory from any species that has not been used hitherto. While bearing in mind the extent to which dried leaves, with flavors that are unusual or even unpleasant to conventional European tastes, are used as relishes in various countries, the palatability of the LP should obviously be assessed before serious work is undertaken.

There is some risk that commercial production as feed for monogastric animals will give LP a stigma. On the other hand, it demonstrates the economic viability of the process. That viability depends on the value of the fibre from which protein has been partly extracted. This contains 65 to 75% water: therefore, between 1.6 and 2.6 t of water have to be evaporated from it to get 1 t of "dried grass" containing 10% water. A good quality crop seldom contains less than 85% water and may contain 90% if harvested early in the day, or in damp weather, so as to keep the drying equipment in continuous use. In these circumstances, the weights of water that have to be evaporated are 5.1 and 8.1 t. Cattle eat the product readily and it has a better feeding value than a crop with the same N content initially. This is because more of the N is true protein and the fibre, being from a less mature crop, is less lignified. Obviously, it contains less protein than the original crop, but a crop such as grass or lucerne, fertilized and harvested so as to give maximum yield, contains more protein than a ruminant needs. It is therefore reasonable to extract the excess for use by people and other non-ruminants. During the extraction of LP, much of the soluble material is removed from the fibrous residue so that it contains less strongly flavored or toxic material than the original crop. Residues from plants such as water hyacinth and potato which animals are unwilling to eat in the fresh state, should therefore be acceptable. This is a point that has still to be established by experiment.

The effluent from silage is well known to be a troublesome pollutant and to kill plants in an area near the silo. There is no effluent from a silo filled with fibrous residue to which just enough deproteinised juice is added to prevent access of air. Silage effluent kills plants because, near the silo, it is too concentrated. If fluid produced during LP production is spread over an area comparable to the area from which the crop was taken, it is a valuable fertilizer. It contains most of the K and much of the N and P of the crop. Ultimately, when there is regular commercial production of LP, it will be used as a culture medium for microorganisms.

Climate and the economic situation of a country influence the manner in which the fractionation of specially grown crops or by-products will fit into the pattern of agriculture. In wealthy countries which need winter feed for cattle, the 2 to 4 fold diminution in the load imposed on a crop drier is the attractive feature: LP would be a by-product. In ill-fed countries hot enough to make artificial drying unnecessary, LP is the primary product and fibre the by-product. Considerations such as these control whether, in designing equipment for fodder fractionation, most attention should be given to maximizing the expression of moisture or the extraction of protein. There should therefore be research in several countries because results in one are not necessarily applicable in others. Whatever policy is being adopted, waste and by-product leaves should get as much attention as leaves from crops grown primarily for fodder fractionation. Widespread interest in making use of wastes is recent. My own interest is not. Thirty years ago (Pirie, 1951) I wrote: "Broadly speaking, anything that has been part of an organism is a potential source of food. Wherever something organic is rotting, smelling, or burning, there is a waste of raw material that could have been turned into food".

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LEAF PROTEIN AS A SOURCE FOR COMBATTING PROTEIN DEFICIENCY

G. Mikeladze, G. Peradze,
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Providing the population with nutritional food products especially those containing protein has become a problem of paramount importance throughout the world.

Conventional methods of obtaining fodder and food protein have proved to be insufficient under the current conditions of the "population explosion" and general shortage of energy resources, thus increasing the level of the protein deficiency.

One of the proposed ways of counteracting protein deficiency is to obtain protein from the green parts of plants. Numerous experiments conducted during the last ten years show that the nutritive value of leaf protein is superior to that of seed protein and is as high as that of fish protein, although lower than that of milk protein. Besides, leaf protein is a good source of β-carotene. However, the use of leaf biomass for protein is prevented by the high content of cellulose and other components. As we know, only 25 of the 300,000 species of higher plants growing on earth are used for food production; therefore, finding a method of extracting protein from the green parts of plants would enable us to utilize more plant species for food.

Originally, research work on methods of preparing protein concentrates from green plants was begun in Hungary and Great Britain. This was followed by a great number of reports on studies carried out in other countries, such as the U.S.A., France, Sweden, the U.S.S.R., etc.

A successful study aimed at obtaining protein concentrates depends very much on the initial material selected, and alfalfa is considered as one of the best plants for this use.

Investigations on alfalfa have been carried out in the U.S.A., Hungary, India, and elsewhere, where its juice is used for the industrial production of protein concentrates. However, there is another way of obtaining protein from the green mass of plants: the use of agricultural wastes which are usually left out in the fields after harvesting, but cannot be used as fodder because they may be toxic. This waste includes the tops of potato, sugar beet, tomato and egg-plants. It would be economically justifiable to extract protein not only from the wastes that cannot be used as fodder, but also from those green parts used as fodder. The output coefficient of the protein consumed by animals does not exceed 0.2 units, the remaining 0.8 units being consumed to maintain the animal organisms. Besides, the losses suffered from using conventional methods of fodder preparation for silage or hay amount to 35-50% (in fodder units per ha.). The losses drop to between 7-10% in the case of hay-meal. However, the artificial drying technique for green fodder is too costly, consuming 0.2 tons of fuel, 150 kW/hr of power, and 183 kW of ABM-1.5A-line installed power per ton of green meal.

Mechanical fractionation ensures a 30% reduction in heat consumption, a 40% reduction in electric power consumption in comparison with grass meal production. Therefore, enriching food and fodder products with protein concentrates obtained from green leaf mass would be more rational than its direct usage as fodder.

The flora and climate of Georgia have the major prerequisites for creating an integrated enterprise for processing green plants. The total amount of green matter for protein production in 1980 was 269,000 tons. The planned output for 1985 is 405,000 tons without taking into account the amount available from wild and pasture grass.

The bulk of green leaf mass is formed during a period of seven months, from May till December, and this reduces the efficiency of the production of protein substances. But this seasonal problem can be overcome by developing hothouse farming, where green leaf mass can be prepared uniformly throughout the year.

We have developed a method of fractionating potato and sugar beet herbage during the course of which the following products were formed: 1) a protein concentrate consisting basically of chloroplast fraction protein and some fragments of leaf cells and containing 40-50% of raw protein; this concentrate can be used as a protein addition to animal diets; 2) a protein isolate consisting basically of cytoplasm fraction protein, containing 80-85% raw protein; 3) the fibrous residue left after juice extraction which contains 15% raw protein that can be used as a substrate for the surface culture of microorganisms. The resulting protein concentrate was green with a distinct grassy smell. The protein isolate was of a light color and had no particular smell.

The optimal pH values of juices for the peak output of protein were found experimentally. The output of cytoplasm fraction protein increased at pH values above pH 7.5.

The chloroplast fraction was isolated by means of controlled heating. A series of experiments was performed in order to find the optimal temperature and duration for thermal treatment. Coagullation by means of controlled heating of the juice took place at temperatures between 30 to 60°C. Changes in the amount of crude protein in the solution were estimated after centrifugation at 1,000 g for 10 minutes.

Another series of experiments was carried out with the aim of studying the effects of time changes on the juice thermal treatment at a given temperature, with controlled amounts of protein nitrogen. The protein nitrogen level was checked because the protein may undergo proteolysis at the time of thermal treatment, which does not affect the calculated crude protein amount, but reduces the content of protein which can be coagulated.

It is clear that a short-term heating followed by a rapid cooling down to 36°C does not change the protein value. In the potato top juice the protein nitrogen value began to drop after 16 minutes' exposure at 56°C, while in the sugar beet top juice it dropped after 20 minutes' exposure at 58°C. After coagulation the chloroplast fraction was isolated by means of centrifugation.

The cytoplasm fraction was precipitated in an acid medium at pH 3.5 and at + 6°C. The precipitate was isolated in the centrifuge. The protein content in the residual juice was measured in order to test the value of the applied methods.

The obtained samples were examined for their amino acid content using the acid hydrolyzate of 6n-hydrochloric acid at 11°C in the amino analyser. As comparative analysis of the essential amino-acid content has shown, the levels of valine, lysine, phenyl alanine, threonine, and isoleucine were higher than in soya meal, alfalfa concentrates and animal proteins. In addition, the obtained samples revealed high levels of glycine, alanine, arginine, aspartic and glutamic acids.

The preliminary experimental results (Table 1) suggest that one ton of potato plant tops can provide 16.7 kg of protein concentrate and 3.2 kg of protein isolate; one ton of sugar beet tops can provide 20 kg of protein concentrate and 4 kg of protein isolate, with 100-120 kg of residual fiber in each case.

It is therefore concluded that the problem of obtaining leaf protein is important both from the scientific and practical viewpoints and it can be produced economically.

Table 1. The amino acid content of the obtained protein preparations

Protein sources	Essential Amino Acids, g/100g of Protein						
	Lysine	Phenyl alanine + tyrosine	Methionine + cystine	Threonine	Isoleucine	Leucine	Valine
Potato top protein concentrate	4.39	3.71	-	4.5	4.5	7.65	6.4
Sugar beet protein concentrate	6.97	-	-	8.09	2.39	2.6	7.6
Potato top protein isolate	3.46	4.69	0.43	4.23	3.73	7.15	5.0
Sugar beet protein isolate	5.25	8.18	2.38	5.95	5.69	10.4	7.18
FAO reference protein	5.44	6.08	3.54	4.0	4.0	7.04	4.96

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MICROBIAL BIOCONVERSION OF PLANT RAW MATERIAL TO PROTEIN-ENRICHED FEED

M.J. Beker

Plant raw material is viewed as a prospective source of food and feeds and as a raw material in a number of industrial applications. Plants provide a renewable resource and it is possible to increase the yield of agricultural crops by applying modern methods of genetics and selection, and by improving agricultural practices.

Under Baltic conditions the yield of crop plant mass may be 10 t/ha total dry mass.

Modern economics requires rational methods of processing the whole biological crop into high quality food and feed products, with a minimum loss of organic substances. This report considers some methods of processing plant green mass, as well as by-products of grain, into protein-enriched products by microbiological procedures.

Green biomass fractionation is aimed at obtaining protein concentrates (Pirie 1968). Green mass is divided in presses into press cake and juice by traditional methods of green crop fractionation. From juice, protein is coagulated thermally and isolated in concentrated form, with a low content of cellulose, as a protein additive to pig and poultry rations, while the press cake with a reduced protein content of 25-35% is used to prepare hay or a finely ground feed.

The liquid fraction - the green juice which makes up up to 50% of the mass processed - may be a source for bioconversion. During anaerobic fermentation of juice, carbohydrates are converted to acids which cause protein coagulation and inactivate saponins and trypsin inhibitors (Stahmann 1976; Beker et al. 1980). Up to 100-150 kg protein concentrate containing about 50% protein is obtained from 1 t dry mass of alfalfa. The yield

of protein concentrate resulting from sugar beet top processing is somewhat lower.

Compared to the thermal coagulation of protein, anaerobic fermentation of plant juices increases by 10-13% the content of methionine in the protein concentrate (Table 1).

Table 1. Comparison of the protein concentrates of alfalfa and sugar beet tops

Protein Concentrate	$\frac{N_{protein}}{N_{total}} .100\%$	Methionine, % protein
Thermal of alfalfa	90.8	1.31
Fermentative of alfalfa	92.0	1.59
Thermal of sugar beet tops	92.8	1.62
Fermentative of sugar beet tops	95.2	1.87

A protein concentrate is isolated as a paste from centrifuges or settling tanks and has a pH of 3.8-4.2 which raises its cost of storage. Dry concentrates are however more suitable for prolonged storage. Biological experiments on chickens demonstrated that acidic protein isolated from sugar beet tops can substitute for 10 to 20% of the protein of full-value rations.

Yeast can be grown on the protein-free brown juice which contains 5 to 7% dry matter and 15 to 20 kg yeast biomass can be obtained per cubic meter, which raises the amount of the obtained proteins by 25 to 30% (Hollo and Koch 1971; Beker et al. 1979).

Another method of applying brown juice after anaerobic fermentation is to obtain organic acid concentrates by evaporating 30-50% dry matter content.

Such a concentrate contains 10 to 20% acid and can be used as a feed preservative, or after enrichment with nitrogen, as an additive to ruminant feed. The anaerobic fermentation of plant juices is attracting wider interest due to the simplicity of the technological process and its low energy consumption. Fermentation may be carried out by a continuous procedure (Beker et al. 1981). We showed that a decrease in the flow rate from 0.1 to 0.03 hr^{-1} changes the ratio of lactic and acetic acids in favor of the latter, which demonstrates the domination of the heterofermentative lactic fermentation over the homogeneous one at low flow rates.

In grain production, roots and stubble make up 25% of the plant biomass, grain makes up 37.5% and straw -37.5% (Bruin 1980). Thus, half of the surface part is the by-product, straw which is rich in cellulose. It is quite evident that straw should become one of the main objects of investigation, namely the bioconversion of its components to more valuable products - proteins, alcohol, biogas, etc.

Another by-product of grain processing is wheat bran - rich in carbohydrates and a prospective raw material for bioconversion. We studied the bioconversion of wheat bran polysaccharides by monocultures, and in association by two producers of amylases and cellulases.

The yeast-like culture Endomycopsis fibuliger R-574 obtained by autoselection from the initial strain Endomycopsis fibuliger R-313 by continuous cultivation, was used as a producer of gluco-amylase, while the culture of the mould fungi Trichoderma lignorum OM 534-6-2 was used as a producer of cellulase.

Combined cultivation was done by submerged and surface procedures. Submerged cultivation was conducted under laboratory conditions in shake flasks and upright fermentors FC-6, while surface cultivation - in cuvettes in a growth chamber at a medium moisture level of 55-60%, a layer thickness of 25 mm and a temperature of 30°C.

It has been established that the combined cultivation of the polysaccharide hydrolase producers gives higher enzyme activities, compared to those achieved in the case of monocultures. At the 72nd hour of submerged cultivation, the glycoamylase activity constituted more than 50 unit/ml, and that of cellulase more than 1 unit/ml, irrespective of the sequence in which the cultures were inoculated.

It is important to note that during the submerged cultivation of Endomycopsis fibuliger, the mycelial form of its development prevails during the first days, and later it develops separate cells in the stationary phase, and the maximum synthesis of glyco-amylase can be observed.

In addition, the biosynthesis of the abovesaid polysaccharide hydrolases by mixed producers was studied during the solid (surface) fermentation of the natural wheat bran medium.

During surface cultivation of Endomycopsis fibuliger R-574 - glycoamylase producer - the culture, on any medium, synthesizes not only glycoamylase, but cellulase as well, and this was not observed during submerged cultivation.

The results of the combined cultivation of Endomycopsis fibuliger and Trichoderma lignorum showed that the maximum cellulase and glycoamylase activity was achieved at the 43rd hour of cultivation in all cases (Table 2). During the combined surface cultivation of the two cultures, an increased

Table 2. Solid-phase fermentation of wheat bran

Culture	Main ingredients of the process								
	Moisture, %	GA, unit/g	C_2, unit/g	Moisture, %	GA, unit/g	C_2, unit/g	Moisture, %	GA, unit/g	C_2 unit/g
	24 hours			43 hours			48 hours		
1. *T. lignorum OM 534-6-2* + *End. fibuliger R-574* (simultaneously)	53.8	230	1.9	35.6	409	6.8	27.0	253	6.8
2. *T. lignorum OM 534-6-2*	54.6	200	2.8	44.0	353	6.8	38.4	195	6.6
3. *T. lignorum OM 534-6-2* + *End. fibuliger R-574* (after 20 hours)	54.0	198	2.1	38.6	394	3.8	35.6	211	1.2
4. *End. fibuliger R-574*	53.4	188	2.1	48.2	334	7.2	48.0	370	6.8

biosynthesis of cellulase and glycoamylase enzymes can also be observed. The fermented wheat bran contains 23-26% protein.

Biological experiments on chickens showed that a 5% substitution of full-value rations by the fermented bran causes a 7-10% increase in the effectiveness of these rations.

It is important to note, also, that the solid-phase fermentation of nonsoluble plant substrates differs from submerged cultivation in that the technological process is simpler and has a reduced energy consumption. Therefore, the described method of microbiological enrichment of starch- and cellulose-containing raw materials with protein can be applied in feed supply units on small-scale equipment, on condition that they are centrally supplied with the necessary seeds for inoculation.

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A MATHEMATICAL MODEL TO MONITOR PRODUCT LOSSES DURING FOOD PROCESSING

D.R. Heldman, and J.P. Norback

Introduction

The opportunities for improvement in efficiency of raw food product utilization are significant when considering the magnitudes of raw product that are not a part of the primary product reaching the consumer. As indicated by Heldman (1979), losses and/or wastes maybe as much as 40 to 60% of the raw commodity when considering food products such as potatoes, beef or apples. Although portions of these losses and/or wastes must be considered intentional, there have been limited attempts to increase magnitudes of raw materials within the primary product or evaluate alternate uses of the waste streams from food processing.

The feasibility for reduction of losses and/or wastes from food processing has improved significantly in the past few years due to two factors: a) rapidly increasing costs of energy and b) increased costs for treatment of waste streams. Costs of energy influence the value of the product at all stages in the food chain and provide justification for recovery of product components that might normally appear in a waste stream. Nearly simultaneously, the laws associated with maintaining water quality have resulted in major adjustments in the cost of waste treatment. By considering both factors, the feasibility of reducing losses and/or wastes as well as recovery of components from waste streams have become popular alternatives for the food industry.

Although mathematical models have become an important component of process analysis in all phases of industrial research, the use of models for monitoring processes has had limited applications in food industry. The application being proposed in this manuscript would suggest the use of mathematical models as tools for decision-making when considering the feasibility of loss and/or waste reduction technologies. Proper formulation

of the model should lead to identification of locations of maximum product loss and the development of the feasible technologies for assuring that maximum quantities of important product components are in primary product streams.

The magnitudes of losses and/or wastes from food processing operations have been summarized by Heldman (1979) and Heldman (1981). These reports indicate that losses from primary product streams are approximately 52% for potato processing, 40% for fresh beef, 50% for apple processing and 3.5% for milk processing. The locations of maximum product loss have been identified for each commodity with peeling and cutting operations contributing most to losses during potato and apple processing. The major losses associated with fresh beef handling are identified as cutting and trimming operations. Processing operations (pasteurization and storage) contribute most to the relatively small lossed during milk processing. Although these results tend to indicate locations where losses are large, the most appropriate steps to be initiated in a loss reduction program can be achieved through a mathematical analysis.

The objectives of the analysis to be presented include:

1. To develop a mathematical model to describe magnitudes of product losses during food processing.

2. To illustrate the use of the mathematical model through application to operations associated with a specific food commodity.

3. To evaluate the feasibility for reduction of food losses and/or wastes during food processing using the mathematical model.

Model Development

A mathematical description of product losses in a sequence of processing operations requires several unique features. These features include:

1. The model should provide the ability to monitor important product components at all stages in a sequence of operations.

2. The description must include the capability to incorporate factors to account for characteristics of each conversion process.

3. The model should lead to a point where losses at given locations in a sequence can be minimized and/or resource recovery can be optimized.

The proposed model is developed in a general manner for a sequence of n operations as illustrated in Figure 1. As presented, the model describes individual conversion operations involving a food commodity and water. For whatever purpose, the

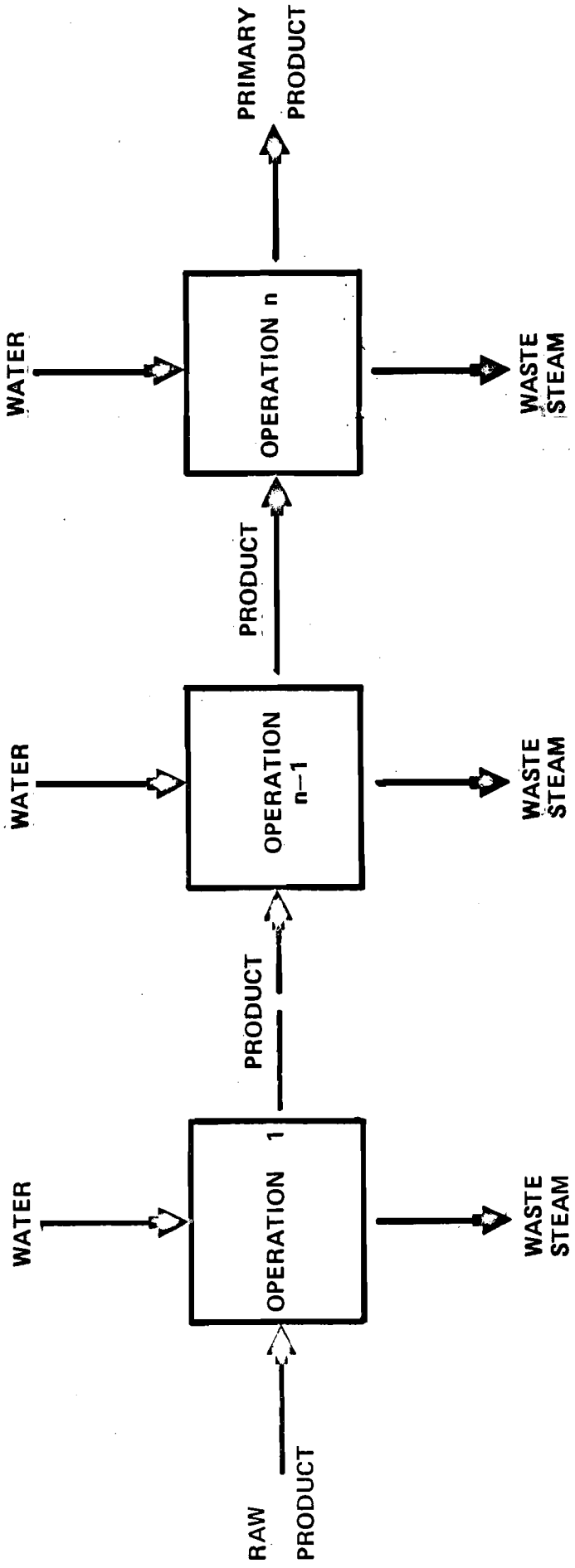

Figure 1. Schematic illustration of the sequence of food processing operations.

product and water are brought into contact during the operation and leave the operation in separate streams. The model describes or monitors the quantities of product that are leaving with the loss stream and not with the primary product stream. The proposed description would allow for several input streams and/or monitoring of several individual product components.

The proposed model utilizes a conversion matrix with the output from a given operation (n-1) expressed as:

$$T_{n-1} = \begin{bmatrix} t_{1k} & t_{1k+1} & t_{1k+2} & \cdots & t_{1k+n} \\ t_{2k} & t_{2k+1} & t_{2k+2} & \cdots & t_{2k+n} \end{bmatrix} \quad (1)$$

where:

t_{1k+1} = composition of product component leaving operation; kg component out/kg product in

t_{1k+n} = water content of product leaving operation; kg product water out/kg product in

t_{2k}, t_{2k+1} = product component in exit water stream; kg product component out/kg water in

t_{2k+n} = water content of water stream leaving operation; kg water out/kg water in

As indicated by the definitions of the various components of the matrix, the upper row values represent coefficients for conversion of product input into primary product output. The bottom row of coefficients indicate product components in the water stream leaving the operation.

The second matrix required for the model is an input matrix defined as:

$$S_{n-1} = [s_1 \quad s_2] \quad (2)$$

where:

s_1 = total mass of product into operation

s_2 = total mass of water into operation

By multiplication of the two metricies, the total output of each component from the operation is obtained. For example, a two component system would be described by:

$$P = S \times T = [s_1 \quad s_2] \begin{bmatrix} t_{11} & t_{12} \\ t_{21} & t_{22} \end{bmatrix} \tag{3}$$

$$P = [s_1t_{11} + s_2t_{21} \quad s_1t_{12} + s_2t_{22}] \tag{4}$$

where:

$s_1t_{11} + s_2t_{21}$ = total mass of product component

$s_1t_{12} + s_2t_{22}$ = total mass of water

In order to use the proposed model to monitor product loss, the outputs from at least two matrix multiplications must be compared. The first can be referred to as Perfect Technology:

$$P^B = S \times T^B \tag{5}$$

where the output would represent maximum possible conversion of input product to primary product as output. The second output would be the Actual Technology with:

$$P^A = S \times T^A \tag{6}$$

and an output representing the actual conversion of product components to primary product. By computation of the difference between the Perfect Technology (P^B) and the Actual Technology (P^A), the losses associated with a given operation are established:

$$\text{Losses} = P^B - P^A \tag{7}$$

The sequential computation of losses as reflected in the reductions of product quantities from one operation to another provides the basis for monitoring product losses in a series of operations. Assuming the appropriate conversion coefficients are known or can be measured, the proposed model will allow monitoring of several product components as the product is converted from raw material to a final primary product.

Application of the Model

As indicated by Heldman (1981), potato processing operations involve numerous individual stages and significant magnitudes of product loss. More recent analysis indicates that the manufacturing of frozen french fries and similar potato products can be described by twelve (12) separate steps where product modifications require water use and corresponding product loss. These operations are described in Table 1. As illustrated, each stage is required to assure that the desired product will be produced by the sequence of operations.

The results in Figure 2 illustrate the magnitudes of product loss at several of the operations. As is evident, the peeling, trimming and sizing operations are major contributors to the total loss of 36.6 kg product/100 kg raw product entering the system. Another significant loss is a part of the liquid effluent which contains product solids from several sources. The composition of the various waste streams will vary depending on the source as indicated by Figure 3. A major portion of the total waste stream is identified as alcohol in soluble solids (22.61 kg/100 kg product solids) while much smaller components are ash and alcohol soluble solids. Reducing sugars were a nearly insignificant protion of the total waste stream. As would be expected, ash was the largest proportion of the silt waste stream. The waste stream from the trimming and sizing operations contained significant amounts of alcohol in soluble solids indicating that portions of potato tubers in the waste streams are contributing to the starch losses.

In order to apply the proposed model to the potato operations described in Figure 3, specific input information is required. Data collected for Figure 3 along with water usage data presented by Shirazi (1979) have been used to develop Table 2. Based on data provided and mass balance on each operation, input values for the conversion matrix have been computed. For this example, the product composition has been treated as two components whereas the model can be used for an unlimited number of product components.

The application of the proposed model first requires computation for Perfect Technology using equation (5). Assuming perfect technology and applying the model to the trimming operation results in:

$$P^B = \begin{bmatrix} 0.225 & 0.775 \\ 0 & 1.0 \end{bmatrix} \text{ X } [100 \quad 4.21] = [22.5 \quad 81.71]$$

Table 1. Operations involved in processing of frozen french fries and similar potato products

Operation	Description
1. Sorting	Removal of visibly unacceptable raw potato tubers
2. Silt removal	Washing of potato tubers to remove major portions of soil
3. Cleaning	Additional cleaning of potato tubers
4. Peeling	Use of steam or other agents to remove peel from tuber
5. Scrubber-Washer	Cleaning of potato tuber after peeling
6. Trimming	Manual removal of unacceptable tubers or tuber portions
7. Cutting	Cutting the potato tubers into portions desired for final product
8. Sizing	Removal of tuber portions that may be too small or too large
9. Blancher I	First stage of operation for enzyme inactivation and texture improvement
10. Blancher II	Second stage of operation to assure low bacterial count and desired color.
11. Dry Handled Waste	An accumulation of product solids from several previous stages
12. Miscellaneous	Additional product losses unaccounted for in other stages

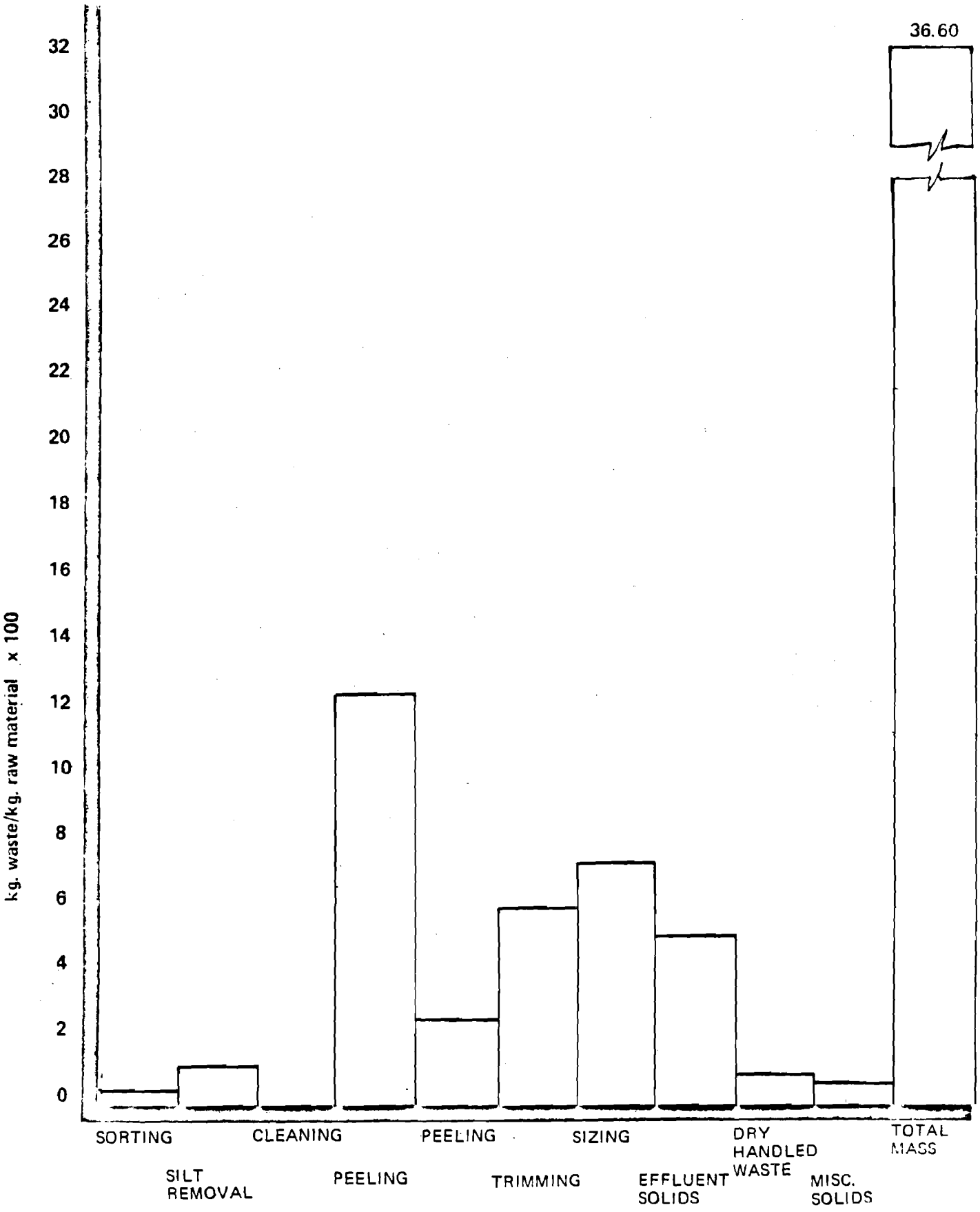
36.60
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
kg. waste/kg. raw material x 100
SORTING
CLEANING
PEELING
SIZING
DRY HANDLED WASTE
TOTAL MASS
SILT REMOVAL
PEELING
TRIMMING
EFFLUENT SOLIDS
MISC. SOLIDS

Figure 2.

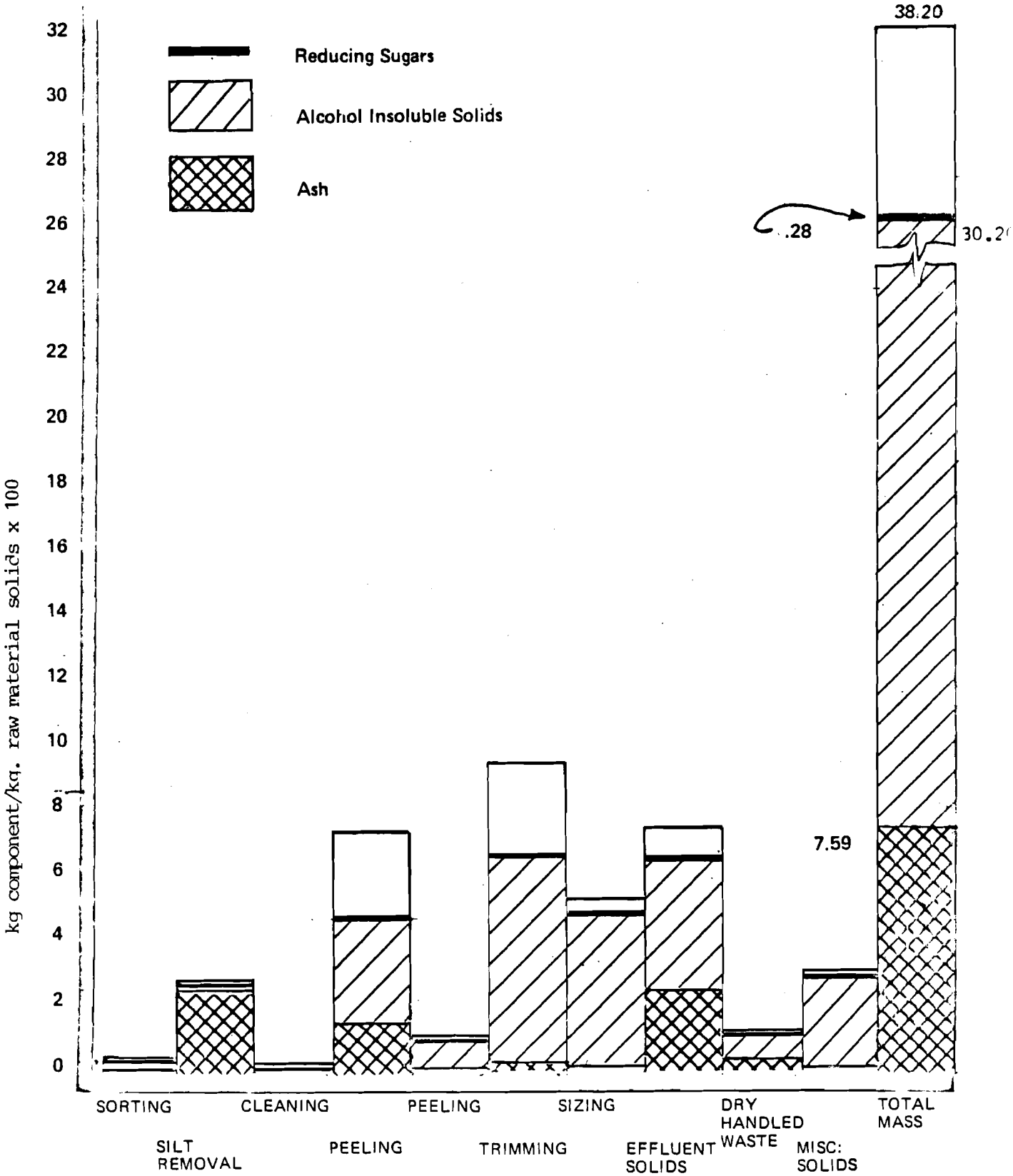
Reducing Sugars
Alcohol Insoluble Solids
Ash
kg component/kg. raw material solids x 100
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
38.20
.28
30.2
7.59
SORTING
CLEANING
PEELING
SIZING
DRY HANDLED WASTE
TOTAL MASS
SILT REMOVAL
PEELING
TRIMMING
EFFLUENT SOLIDS
MISC: SOLIDS

Figure 3.

Table 2. Product loss and water usage for processing of frozen french fries and similar potato products

Operation	Water Usage (kg H_2O/kg prod.)	Waste Stream Solids (kg solids/100 kg)	t_{21} (kg solid/kg H_2O)	t_{22} (kg H_2O/kg H_2O)
Sorting	0.1445	0.45	0.0070	1.024
Silt removal	0.0437	2.89	0.1487	1.513
Cleaning	0.0208	0.10	0.0108	1.037
Peeling	0.3844	8.43	0.0490	1.170
Scrubber-Washer	0.8684	9.07×10^{-9}	0.0	1.0
Trimming	0.0421	9.40	0.5020	2.730
Cutting	0.2578	2.07×10^{-8}	0.0	1.0
Sizing	0.1974	5.28	0.2131	0.734
Blanching I	0.4931	8.77×10^{-10}	0.0	1.0
Blanching II	0.7018	5.60×10^{-10}	0.0	1.0
Dry Handled Waste	0.0100	1.09	0.2450	1.845
Miscellaneous	0.9042	2.98	0.0074	1.026

This computation indicates that the composition of the potato (22.5% solids, 77.5% water) is maintained during the conversion process. The input matrix was set up using 100 kg product in and 4.21 kg water as obtained from Table 2. The output matrix indicates that 22.5 kg product solids has been preserved and the total quantity of water leaving is 81.71 kg. This quantity of water includes 77.5 kg in the product and 4.21 kg water provided for the conversion process.

The use of the model for the actual technology process requires recognition that the model assumes conservation of mass and that losses are reflected in transfer of product solids from primary product to liquid waste streams. If follows that:

$$P^A = [22.5 \quad 81.71] = [100 \quad 4.21] \times \begin{bmatrix} t_{11} & t_{12} \\ 0.502 & 2.73 \end{bmatrix}$$

where the values of t_{21} and t_{22} have been computed from data presented in Table 2. The model is then used to compute:

t_{11} = 0.2039 kg solids out/kg product in

t_{12} = 0.7022 kg water out/kg product in

Based on these computations, several observations are possible. First of all, the loss of product solids is 0.0211 kg/kg product (0.225 - 0.2039) indicating the quantity of product solids that would be required to compensate from losses in the trimming process. In addition, the total product loss of 0.0939 kg/product entering (1.0 - 0.9061) can be determined.

The values of conversion matrix components as well as magnitudes of solids and product loss for each of the twelve potato processing operations is presented in Table 3. As is evident, the magnitudes of the coefficients (t_{11}, t_{12}) vary significantly with operation. The lower quantity coefficients are associated with operations having more significant product losses as indicated by additional data presented on the table. The solids loss and product loss values should be viewed as quantities needed to compensate for losses with recognition that the type of solids or product required to replace losses will change with location in the sequence of operations. For example, losses during sorting or cleaning can be replaced by potatoes or potato solids in the same condition as those that are lost at this point in the sequence.

Table 3. Product losses computed from conversion matrix for processing of frozen french fries and similar potato products

Operation	t_{11}	t_{12}	Solids Loss	Product Loss
	(kg solids out/kg prod.in)	(Kg H_2O out/kg prod.in)	(kg solids/kg prod.)	(kg prod./kg prod.)
Sorting	0.2240	0.7715	0.0010	0.0045
Silt removal	0.2185	0.7526	0.0065	0.0289
Cleaning	0.2248	0.7742	0.0002	0.0010
Peeling	0.2060	0.7097	0.0190	0.0843
Scrubber-Washer	0.2250	0.7750	0.0	0.0
Trimming	0.2039	0.7022	0.0211	0.0939
Cutting	0.2250	0.7750	0.0	0.0
Sizing	0.2131	0.7340	0.0119	0.0529
Blanching I	0.2250	0.7750	0.0	0.0
Blanching II	0.2250	0.7750	0.0	0.0
Dry Handled Waste	0.2226	0.7666	0.0024	0.0108
Miscellaneous	0.2183	0.7519	0.0067	0.0298

Another use of the proposed model would be as a sequence of operations using the coefficients and matricies to compute outputs from each operation. The first operation would appear as:

$$P^A = \begin{bmatrix} 0.224 & 0.7715 \\ 0.007 & 1.024 \end{bmatrix} \times [100 \quad 14.45] = [22.5 \quad 91.95]$$

indicating that all product solids and water entering the first operation (sorting) have been accounted for. Using the fact that 0.45 kg product/100 kg product in have been lost in the first operation, the second operation (silt removal) would be:

$$\begin{bmatrix} 0.2185 & 0.7526 \\ 0.1487 & 1.513 \end{bmatrix} \times [99.55 \quad 4.35] = [22.399 \quad 81.503]$$

These computation indicate that product solids leaving the second operation include 22.399 kg in both the primary product and the liquid waste stream. By continuing similar computations in sequence, the final operation would be:

$$\begin{bmatrix} 0.2183 & 0.7519 \\ 0.0074 & 1.0255 \end{bmatrix} \times [73.206 \quad 66.193] = [16.471 \quad 122.925]$$

revealing that product solids converted in the final operation is 16.471 kg on the basis of 100 kg entering the sequence of twelve operations.

In addition to the monitoring of solids losses and product losses and the ability to indentify quantities and types of product required to compensate for losses, the proposed model can be used to evaluate factor influencing magnitudes of losses. By varying the water usage to individual operations, the influence on losses can be evaluated as a first step in determining operations deserving more in-depth analysis. In addition, the impact of feasible reductions in waste stream solids could be evaluated efficiently.

The proposed model has two additional characteristics that would be useful in evaluating potential for reduction of product losses. The model has the capability to incorporate a complete

array of product components. This allows monitoring of all product components and the possibility of selecting operations for more detailed analysis on the basis of specific component recovery. The second characteristic of the model is the ability to incorporate optimization. This step would allow more detailed analysis of alternatives that might result in more effective utilization of raw product resources.

CONCLUSIONS

1. A mathematical model incorporating a conversion matrix and an input matrix has the capability to monitor product components during typical food processing operations.

2. Applications of the proposed mathematical model to potato processing operations leads to computation of coefficients for the input matrix from typical water usage and solid loss data.

3. The proposed model is capable of monitoring product losses in a sequence of operations and allows for evaluating loss reduction potential.

4. The potential for monitoring all product components through a sequence of operations is possible with the model along with the opportunity for optimization of product solids recovery.

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