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FEASIBILITY OF PRODUCING A RANGE OF FOOD PRODUCTS FROM A LIMITED RANGE OF UNDIFFERENTIATED MAJOR FOOD COMPONENTS

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I. INTRODUCTION

In considering extended manned space missions it is necessary to evaluate the potential for regenerative life-support systems, including those with limited as well as complete closure. Analysis of problems related to nutrition, diet, and food processing showed that a number of long-range research studies will be required if a fully regenerative food ecology is to be developed. The questions to be answered in such a long-range program include the following broad catagories (each being a parent to a host of subsidiary questions):

A. Nutritional requirements of humans on a limited variety diet, especially while operating under conditions of space environment.

B. Capability of "agriculture", waste disposal technology and subsidiary technological operations to attain a closed-cycle operation adequately supplying the life support needs for the expected population.

C. Need for buffering capacity (storage or resupply capability) to accomodate expected or unexpected breakdowns in regenerative capabilities.

D. Development of a base for conversion of plants, animals, and synthetic materials into "food".

The aim of food production and food processing systems is to have a reliable supply of food, that is capable of providing satisfactory nutrition in an acceptable form. This task is more complex than simply replicating, on a smaller scale, the food pattern of the United States.

The constraints and considerations inherent in the technical development, formulation, and provision of food items for the large scale commercial market are not necessarily compatible with major criteria necessary for foods for a closed ecological life supply system. Most commercial food items are designed, deve-

'oped, and formulated to be suitable for mass production and mass distributions with relatively rapid turnover in the distribution system.

To provide an acceptable and varied food supply for the limited facilities available in the environment of space habitats in conjunction with dietary requirements is a difficult task. Moreover, the need to optimize nutrient balance and minimize food system mass, while providing an appetizing diet of variety and quality even remotely similar to that which we enjoy on earth, presents immense difficulties.

An analysis of the potential problems of food processing in space has been prepared (Karel, 1980). Subsequently, we have conducted under NASA sponsorship an evaluation of the requirements of a system in which conventional agriculture using a limited number of plants and resupply of animal products formed the base for the diet of the inhabitants of a space habitat.

We believe that the provision of engineered foods which are produced by incorporation of natural and/or synthetic components into systems having desired nutritional organoleptic and stability characteristics is feasible.

The development of knowledge about functionality of ingredients provides the basis for development of engineered foods. Since organoleptic properties are by far the most important stimuli for making nutrients into food, development or organoleptic equivalence of engineered foods allows utilization of a variety of nutrient sources no matter what their origin. Furthermore, organoleptic equivalence also permits the construction of nutrient sources to the specification of human need rather than dependence upon the vagaries of natural products.

Engineered foods have specific advantages in the applications to space habitat diets. Flexibility is an obvious advantage, since it will allow various levels of incorporation of space-grown food ingredients into the engineered food system (if

-t least part of the food preparation is done in the habitat), will allow choice of components particularly to resupply (because of stability, palatability or other considerations) and will allow modularization of the diet which could be invaluable in case of changes in requirements or in supply.

Over the last several years experts in the field of life support in space held a number of workshops, in many of which the present author participated. It is clear that the number of persons to be stationed in a space habitat, the environment of the habitat, the capabilities of periodic resupply, the type of vegetation to be harvested and many other key factors in the CELSS scheme have been continually revised.

II. OJBECTIVES

The development of a feasible design for the food system within a CELSS will require entirely new concepts of production, processing, preparation of foods and of waste disposal. This development will require cooperative work sustained at least over the next decade, involving experts in the fields of plant growth, ecology, chemical and industrial engineering, nutrition, medicine and food technology. Our major objective is to contribute food technology expertise to fabricate safe, nutritious, and acceptable "food" within the constraints of a scenario for CELSS, which seems reasonable at present. The immediate task is to assess the "Feasibility of producing a range of food products from a limited range of undifferentiated major food components". The underlying assumption which is used in this review includes the production of macronutrients, i.e. proteins and/or amino acids, polysaccharides and/or monosaccharides, lipids, and/or glycerol.

The specific source of these raw materials may vary as the CELSS scenaria change. An assumption of production of wheat and soy would readily fit the needs of the review model, but we shall not exlude the possibility that synethsis or biosynthesis of specific components (e.g. glycerol, monosaccharides) or microbial or algal sources of protein and/or fat will be used.

III. ASSUMPTIONS

1. Knowledge of the nutritional requirements of the space crew in CELSS is an essential requirement for the design of all food-related activity regarding the space habitat. We assume that the recommendation of the Food and Nutrition Board, National Academy of Sciences, National Research Council regarding the daily dietary allowances (RDA) for a normal, healthy man on earth is suitable for space needs (Table 1).

2. Since the "nutrients" are necessary for long-term maintenance of health and work efficiency and "foods" are the vehicle for them, the produced undifferentiated nutrients are therefore fabricated into "food" which should provide a "safe", nutritionally "adequate", and at least marginally "acceptable" diet for the crew members. Attempts should be made to approach the maximum Index of Nutritional Quality , INQ, (Hansen et al., 1979) in order to minimize the production load and waste treatments. Crew members are assumed to be already conditioned for consumption of such fabricated foods for long periods of time. Consequently, the consumption of such foods will not cause any psychological or social problems for them in the space habitat.

3. In order to produce the most balanced diet, energy distribution from macronutrients (proteins, lipids, carbohydrates) and their distribution in various food groups will be adjusted according to the dietary goals for the United States released by the Senate Select Committee on Nutrition and Human Needs (Petarkin, 1978) as follows:

Table 1

Recommended daily dietary allowances (for a normal, healthy man aged 23-50 years with an average weight of 70 kg and average height of 178 cm) by Food and Nutrition Board, National Academy of Sciences, National Research Council (NAS and NRC, 1980)

Energy	2700 Kcal
Protein	56 g
Vitamin A	1000 µg RE[a]
Vitamin D	5 µg[b]
Vitamin E	10 mg α-TE[c]
Ascorbic Acid (Vitamin C)	60 mg
Folacin	400 µg
Niacin	18 mg
Riboflavin (Vitamin B_2)	1.6 mg
Thiamin (Vitamin B_1)	1.4 mg
Vitamin B_6	2.2 mg
Vitamin B_{12}	3 µg
Calcium	800 mg
Phosphorus	800 mg
Iodine	150 µg
Iron	10 mg
Magnesium	350 mg
Zinc	15 mg

[a]Retinol equivalents. 1 retinol equivalent = 1 µg or 6 µg beta-carotene.

[b]As cholecalciferol. 10 µg cholecalciferol = 400 IU vitamin D.

[c]α-Tocopherol equivalents. 1 mg d-α-tocopherol = 1 mg α-TE.

Distribution of Energy from Nutrients

- Carbohydrates 58%
  - sugar 15%
  - complex C.H.O. 45%
- Proteins 12%
- Lipids 30%
  - saturaged 10%
  - others 20%

Energy Distribution in Selected Food Groups (%)

	C.H.O.	Protein	Fat
Vegetables, fruits	86	9	5
Cereal, bread	80	12	8
Milk, cheese, ice cream	30	21	49
Meat, poultry, fish	2	36	62
Eggs	2	33	65

4. Pure water in quantities sufficient for production, processing and consumption is assumed as provided through "waste treatment" in the space habitat. Macronutrients are produced from the minimum variety of plants. For this purpose soybean and wheat are hydroponically or aeroponically cultured while single cell components are produced from culture of an algae species, e.g. Spirulina (substitution of other single cell systems is possible). Glycerol, and possibly some fatty acids, will be synthesized from simple chemicals generated by waste treatment. As sweetener and

part of energy-producing carbohydrate, cellulosic material produced from soy and wheat culture will be converted to glucose.

5. In addition to the above fabricated foods, wheat and soybean may also be used for their respective conventional products. In such cases, all products made from wheat will use "whole-wheat flour" prepared as in standard wheat milling technology. This decreases the solid waste and needed equipment. For oil extraction from soybeans an aqeuous extraction (to minimize chemical use) is assumed. However, no animal products, fruits and/or vegetables are produced in the space habitat. Attempts are made to simulate these food products by fabrication of macronutrients into engineered foods.

6. Micronutrients (minerals and vitamins) are partially provided through utilization of macronutrients. Other required micronutrients (deficiencies as well as extra requirements because of environmental stresses) are presupplied. Food-processing additives and some miscellaneous food items (e.g. coffee or tea) are also initially supplied (for the entire mission period) in moisture-free, freeze-dried form.

7. The total population of the space habitat is assumed to be 10 people and the minimum life of the space habitat is 10 years. These figures average out the results of recent transportation analysis for CELSS compiled in Table 2 (Guston and Vinopal, 1982). The gravity is assumed to be partial to almost nil (microgravity). For food production and processing calculations, the unit man-year is assumed.

8. Raw materials that are rengerated in the space habitat "farm" and delivered to the storage room are pre-cleaned.

However, all the raw materials which enter the plant are cleaned (selective sieving, aspiration, magnetic separation, washing, etc.) and wastes of the cleaning steps as well as non-usable by-products will be delivered to waste treatment.

Table 2
Possible Future Manned Space Missions
Data Compiled from Transportation Analysis for CELSS
(Gustan and Vinopal, 1982)

Mission	Number of Inhabitants	Breakeven time for 97% Food Closure
1. Low Earth Orbit (high & low inclination)	4	9.5 - 10 years
2. Geosynchronous	4	13 years
3. Lunar Base	12	10 years
4. Asteroid Base	5,000	3 years
5. Mars Surface Exploration	8	never

9. Processed foods leave the pilot plant to the "finished product" storage area. These storage areas have as stock at all times, foods needed for 6 months of consumption as a reliable "buffering capacity" of the food system. Packages for storage of these processed foods are initially supplied from Earth, and will be light, air-tight and re-usable.
10. There is no waste due to "spoilage".

Figure 1 summarizes the production and processing of nutrients in CELSS.

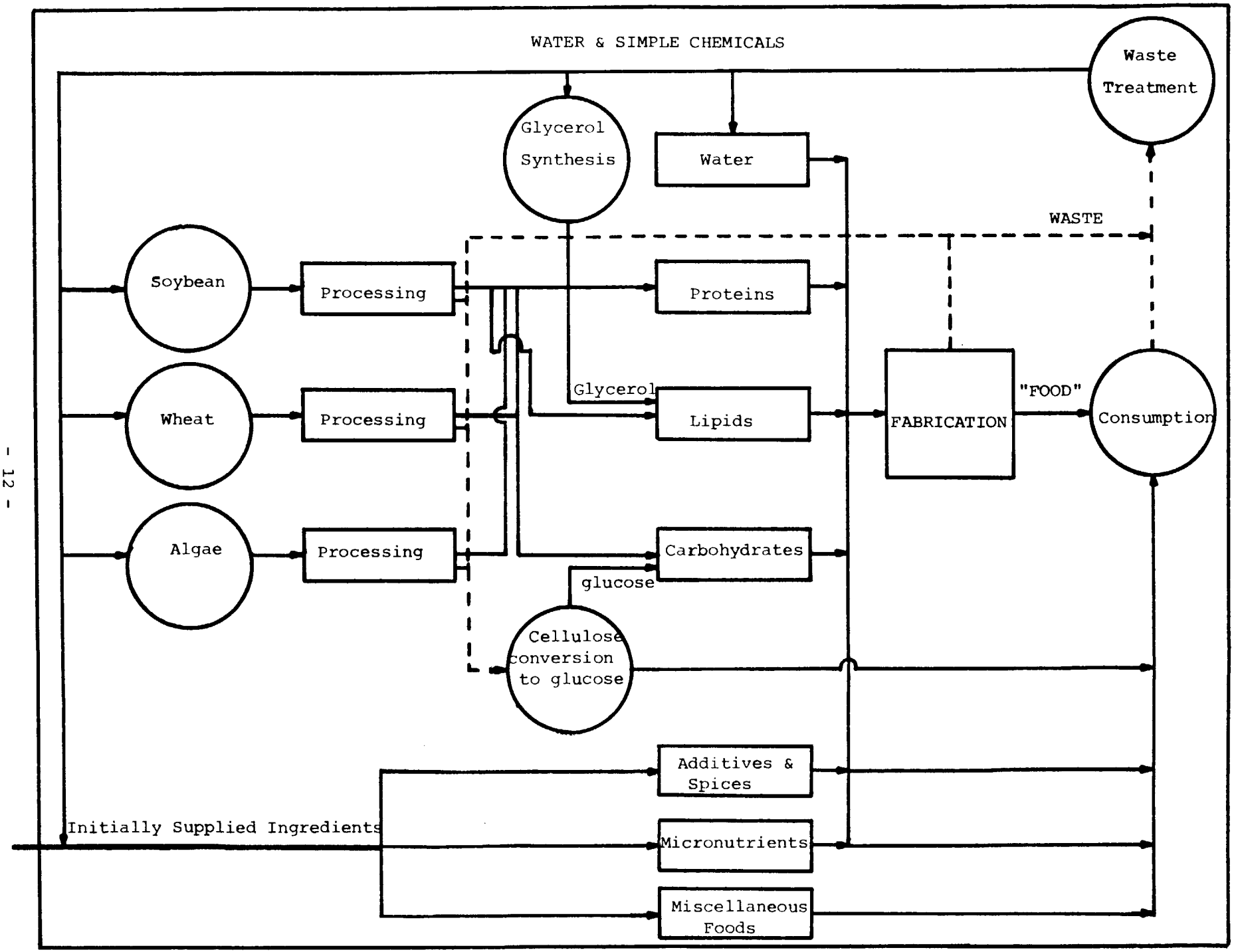

Fig. 1. Overall schematic presentation of food production and processing for a space habitat (CELSS) 10 habitants, 10-year mission.

IV. NUTRITIONAL REQUIREMENTS

Many food elements are needed to make up a diet which is nutritionally adequate. No single natural food supplies them all. Moreover, the nutritional requirements are different for the two sexes. They change with age; they vary with weight, activity, and environment. Individual differences in metabolism have a profound effect upon the utilization of various food elements. Hence, good nutrition is largely a matter of selecting those foods which together add up to meet the requirements of the individual in all the essential food elements (NAS and NRC, 1980). Important to good nutrition is also proper processing, preparation, and cooking foods so as to conserve their nutritive values.

There are still several nutrients naturally present in foods that are known to be required in the diet of certain other animal or microbial species but for which little or no evidence of dietary essentiality exists for humans. There are other substances in foods that in the lay literature are claimed to be nutrients for humans although there is no real evidence for such claims (NAS and NRC, 1980). It is, however, important to note that as fabricated foods from a narrow range of plant products are being substituted increasingly into the diet in the space habitat, the danger of missing some trace elements or an unidentified nutrient also increases (Billingham et al., 1979). Nutritional requirements for CELSS could differ from those of similar terrestrial populations, because of effects from zero or some fraction gravity in the habitat. The astronauts on Skylab, for instance, showed losses of body mass, water, calcium, and electolytes, continuing even after months in space (Billingham et al., 1979).

Precise information is therefore needed on the individual daily requirements of nutrients for space habitat personnel

before an entirely adequate selection can be made of basic sources of these nutrients. These sources of foodstuffs must be known before the sophisticated existing technology of food processing can be fully utilized to optimize nutrition.

In Gemini missions the calorie intake was 2,500 Kcal/day, compared to 2,800 Kcal/day for Apollo and 3,200 Kcal/day for the lunar excursion module (Klicka et al., 1967). Calorie intake has been modified to 3,000 Kcal/day for the space shuttle (Bourland et al., 1977).

In the Skylab program, one of the basic objectives was to learn about human physiology in null gravity. The Skylab menu, therefore, provided specific quantities of each of the following six nutrients: Protein at levels 90 to 125 g/day; whatever the level selected for any one individual, the day-to-day variations are less than $\pm$ 10g. Calcium was 750 to 850 $\pm$ 16 mg, sodium 3,000 to 6,000 $\pm$ 500 mg, magnesium 300 to 400 $\pm$ 100 mg and phosphorus 1,500 to 1,700 $\pm$ 120 mg (Stadler et al., 1973; Heidelbaugh et al., 1973).

This degree of control of nutrients required closed cooperation between food technologists and nutritionists, a cooperation which is necessary whenever any food system is designed to meet the nutritional needs of the consumers (Heidelbaugh et al., 1973; Leveille, 1972).

As indicated under assumptions (III-1), for the nutritional needs for CELSS inhabitants, we literally accept the present RDA for a normal healthy man on Earth (Table 1).

V. PRODUCTION OF MACRONUTRIENTS

In this section we consider the conversion of mostly edible products of hydroponically- or aeropinically- grown plants (wheat and soybean) and one alga (Spirulina), to their constituent macronutrients. We also consider synthesis of glycerol and conversion of cellulosic by-products to glucose. The proposed sources and the obtainable macronutrients in CELSS are summarized as follows:

Source	Macronutrient
wheat	starch, protein
soybean	protein, oil
algae	protein
synthesis	glycerol
cellulose	glucose

Although in the present study we only focus on extraction and utilization of the above macronutrients, consideration should nevertheless be given to other constituents which could have nutritional significance. Crude fiber, vitamins, and minerals of the above plants might prove to be significant factors under CELSS conditions where the availability of these nutrients might be limiting or marginal. The significance of the crude fiber content of the diet has received considerable attention of late because of its possible role in reducing the incidence of colon tumors (Leiner, 1977). Particle size, density, hydration capacity and ion exchange capacity are the four major physical properties of dietary fiber which may play a role in the rheology of a food product.

The macronutrients in CELSS are delivered, along with regenerated water and initially-supplied food additives and micro-

-utrients to the fabrication section of CELSS for conversion to fabricated "food" for consumption by the crew.

A. Macronutrients from Wheat

The edible portion of the total wheat biomass is high (Tibbitts and Alford, 1980). Wheat contains a high starch content, contains a reasonable amount of protein (to 14%), as well as phosphorus, iron, thiamin, niacin and fiber (Adams, 1975). Structurally, the wheat kernel may be divided into 3 main parts (Shurpalekar and Rao, 1977):

1. The germ (embryo) which produces the new plant, comprises 2-3% of the kernel. Wheat germ is the richest known source of tocopherols of plant origin and also a rich source of thiamin, riboflavin, and niacin. However, because of the relatively small quantity of wheat germ production, we do not consider it to be a source of macronutrients in the space habitat. It can, however, be used in fabricated foods.

2. The bran consists of the various outer coverings which protect the kernel and account for 14-16% of it, and is very rich in fiber, calcium, phosphorus and iron. It contains also significant amounts of thiamin and riboflavin and is especially high in niacin. The aleurone layer has high content of ash, phytate, phosphorus, fat and niacin. Thiamin and riboflavin contents in the aleurone layer are higher than in other parts of the bran (MacMasters et al., 1978). In this study we do not consider the bran as a source of macronutrients. However, like germ, it could be used in the production of fabricated foods.

3. The endosperm provides food for the new plant when the embryo starts to grow. The endosperm accounts for 81-84% of the kernel. The wheat endosperm has a cellular structure

and each cell is filled with starch granules, contained within thin cellulose walls, varying in diameter from 1 to 40 microns. Between these starch granules is a proteinaceous material which contains, in addition to proteins, the minerals, coloring matter, and enzyme of the endosperm (MacMasters et al., 1978; Olkku and Rha, 1978). The composition of endosperm varies considerably depending on the variety and milling operations (Table 3).

Table 3
Composition of Wheat Endosperm
(adapted from Inglett, 1973)

Starch	62.7 - 71.7%
Crude protein	7.9 - 16.0%
Lipid	1.6 - 2.2%
Reducing sugars	1.6%
Pentosans	1.4%
Cellulose	0.3%
Ash	0.5 - 0.8%

Starch, the principal carbohydrate of the kernel is found exclusively in the endosperm. Proteins occur in all tissues of the grain, but gluten, the protein we are interested in, is found in endosperm. Wheat lipids are found in low percentage levels and therefore will not be considered for extraction and utilization as energy-producing nutrient. For our purpose, wheat should initially be milled into flour. In the milling operation, the relative order and structure of the endosperm cell is largely disrupted.

Breaking and reduction (grinding) are the most important operations in the milling process. In breaking, the grain is opened up and the contents spilled or released so that they may be separated from the bran layers by means of sieves for subsequent crushing. The opening-up operation requires a combination of pressure and shear, but shattering of the bran must be minimized, since the extent to which bran fragments may be separated from the endosperm after breaking varies inversely with the particle size of the mixture; for the same reason, the endosperm is desirably released mainly in the form of large particles. The breaking rolls are pairs of spirally fluted, chilled iron rolls driven at different speeds (Ziegler and Greer, 1978; Harrel, 1964). Reduction rolls are usually smooth, also rotating together with a speed differential; and their function is to reduce the particle size so that 20-60% of the flour has a particle size of 15-40 microns (Ziegler and Greer, 1978; Harrel, 1964).

a. Wheat Starch

Carbohydrates, forming about 83% of the total dry matter, include starch, which preponderates, cellulose, hemicellulose, pentosans and soluble sugars which include monosaccharides (mainly glucose and fructose) and oligosaccharides such as sucrose, glucofructans, raffinose, maltose, and neoketose (Cerning and Guilbot, 1973). Starch is a reserve polysaccharide laid down in the plant in the form of water-insoluble particles, the starch grandules. American wheat varieties contain from 61.2% to 71.9% starch (Cerning and Guilbot, 1973). Chemically, starch is an α-glucan composed of two components: amylose (an essentially linear chain of anhydroglucopyranose residues jointed by 1,4-α-D-glucosidic bonds) and amylopectin (a branched structure). Molecular weight of 1.1 to 1.9 million for amylose

'Banks et al., 1973; Greenwood, 1956; Killion and Foster, 1960) and 10 to over 200 million for amylopectin (Banks et al., 1973) have been reported. Starch contains varying concentrations of amylose and amylopectin. Wheat starch has an average apparent amylose content of 23% and contains minor constituents (nitrogenous and phosphorus compounds; lipids; and various minerals). Wheat starch is present in two types of granules: large lenticular granules (most with a diameter of 20 to 35 μ) and small spherical granules (diameters in the range of 2 to 10 μ)(D'Appolonia 1971; Guilbot 1963). Although the larger granules represent only 12.5% of the total number, they account for most of the weight and the larger portions of the surface. The properties of wheat starch and their sensitivity to enzyme action depend on the plant source, growing conditions, and maturity (Greenwood 1972).

Preparation of Starch

Radley (1953), Anderson (1967) and D'Appolonia et al. (1971) have reviewed methods of isolating wheat starch. The older methods were largely concerned with recovery of the starch, with little or no thought given to possible use of the gluten. The essential purpose of the Halle fermentation process, for example, was to destroy as much of the gluten as possible so as to free the more resistant starch from the wheat. For our study, however, we need to focus on simultaneous maximum extraction of starch and gluten, since both of these macronutrients will be used as ingredients for food fabrication in CELSS. For example, using the Alsatian process (wet-milling process), it is possible to separate gluten from starch. We consider here only processes which do not require the use of chemicals. In the Alsatian process, the grain is softened by prolonged steeping during which

fermentation is avoided by frequent changes of the steeping water.

In the Martin process (Knight, 1965), a stiff dough containing about 40% water is prepared and allowed to stand 1 hour while the gluten hydrates and swells. The mass is then kneaded under a spray of water to wash the starch from the cohesive mass of gluten. The starch may be purified in a number of ways, one of which is by sieving the slurry to remove any pieces of gluten whic have broken away from the main mass, following by centrifuging and washing. The resulting starch cake could then be dried. During the refining of the starch, a tailings starch of higher protin content than the prime material is usually obtained. It is dried in the same fashion as the prime starch. The batter process differs from the dough method primarily in that a slack dough or bater is produced and agitated with larger amounts of water. The gluten is broken up into small lumps from which the starch slurry is removed by screening. To select the method of choice for starch preparation under the CELSS constraints, comparison of these methods under simulating space conditions is required.

b. Wheat Proteins

Wheat contains 10-14% protein. In wheat endosperm at least 70 or 80 different proteins can be separated, and the total numer of proteins is probably much greater than the number that can be distinguished by available methods (Bushuk and Wrigley, 1973). The insoluble protein part of the endosperm is called gluten which represents 85% of the endosperm proteins (Sarkki, 1979; Kasarda et al., 1978). Table 4 shows the fractionation of wheat proteins based on their solubility (Residue proteins are mainly high molecular weight glutenins).

Table 4

Solubility Fractionation of the Proteinaceous Material in a Typical Straight-Grade Flour from Hard Red Spring Wheat

Solvent	Protein	Amount (%)
Water	Low molecular weight	5.3
Water	Albumin	11.1
0.5 N NaCl	Globulin	3.4
70% Ethanol	Gliadin	33.2
0.05N Acetic Acid	Glutenin	13.6
--	Residue protein	33.4

Unique structural and adhesive characteristics of wheat gluten (as seen in bread dough) and its water absorption properties makes it a suitable and interesting protein for food fabrication under CELSS conditions. In the present study, we focus primarily on "gluten" as the major wheat protein macronutrient.

The separation process of gluten from the other constituents of wheat flour was described in the previous section and is essentially accomplished by physical means from aqueous flour suspensions. In its freshly extracted, wet form it is known as gum gluten which, when dried, yields a cream-to-tan colored, free flowing powder with a bland taste. When rehydrated, it regains its original characteristics. Typical analysis of wheat gluten is presented in Table 5.

Table 5

Typical Analysis of Wheat Gluten

Protein (N x 5.7) dry basis (d.b.)	75.0%-80.0%
Moisture	5.0%-8.0%
Ether extractable fat (d.b.)	0.5%-1.5%
Ash (d.b.)	0.8%-1.2%
Water absorption capacity	150%-200%

The amino acid composition of wheat gluten constituents (gliadin, glutenin and residue protein) is presented in Table 6.

Table 6

Amino Acid Composition of Wheat Gluten Constituents (g per 16 g N) from Bushuk and Wrigley, 1973

	Gliadin	Glutenin	Residue Protein
Tryptophan	0.7	2.2	2.3
Lysine	0.5	1.5	2.4
Histidine	1.6	1.7	1.8
Ammonia	4.7	3.8	3.5
Arginine	1.9	3.0	3.2
Aspartic acid	1.9	2.7	4.2
Threonine	1.5	2.4	2.7
Serine	3.8	4.7	4.8
Glutamic acid	41.1	34.2	31.4
Proline	14.3	10.7	9.3
Glycine	1.5	4.2	5.0
Alanine	1.5	2.3	3.0
Cystine (half)	2.7	2.2	2.1
Valine	2.7	3.2	3.6
Methionine	1.0	1.3	1.3
Isoleucine	3.2	2.7	2.8
Leucine	6.1	6.2	6.8
Tyrosine	2.2	3.4	2.8
Phenylalanine	6.0	4.1	3.8

The amino acid composition of gluten is characterized by unusually high contents of glutamine and proline. Of nutritional importance is the relatively low level of lysine, the most limiting of the essential amino acids. After lysine, tryptophan

and methionine also tend to be nutritionally limiting. Doguchi and Hlynka (1967) showed that differences in dough properties between several flour types were largely reflections of differences between the glutens washed from the flours. By way of contrast with conclusions of this type, other workers have claimed that glutens with similar quality characteristics were washed from different types of flour (Webb et al., 1971). Their main point was to emphasize that the quantity of gluten in a dough overrides any consideration of its intrinsic quality.

The ability of wheat gluten to form a viscoelastic mass when fully hydrated sets it apart from other vegetable proteins. The major reason for the ability of gluten to form an elastic, coherent matrix is the tendency of gluten proteins to interact and associate with one another (Bushuk and Wrigley, 1973). Glutenin and gliadin interact in an aqueous system to produce this viscoelastic property. Glutenin contributes elasticity and gliadin provides extensibility. These properties are summarized in Table 7.

Table 7
Fractionation of Wheat Gluten

Gliadin	Glutenin
Highly extensible	Less extensible
Less elastic	Highly elastic
Soluble in alcohols	Insoluble in alcohols
Low molecular weight (less than 100,000)	High molecular weight (greater than 100,000)
Intramolecular bonds	Intra & intermolecular bonds

The ability of gluten to form adhesive and cohesive masses, films, and three dimensional networks, is crucial in fabrication processes.

The film forming property of hydrated gluten is a direct outcome of its viscoelasticity. Whenever carbon dioxide or water vapor forms internally in a gluten mass with sufficient pressure to partially overcome the elasticity, the gluten expands to a spongy or cellular structure. In such structures, pockets or voids are created which are surrounded by a continuous protein phase to entrap and contain the gas or vapor. This new shape and structure can then be rendered dimensionally stable by applying sufficient heat to cause the protein to denature or devitalize and set up irreversibly into a fixed moist gel structure or to a crisp fragile state, depending on the final moisture content.

B. Macronutrients from Soybean

The inclusion of soybeans as a source of macronutrients provides a plant type that is representative of other legumes that might be utilized in space habitats. Soybeans can serve as a major source of protein and oil which are needed for food fabrication in the CELSS. Soybeans are well known for variations in physical properties, as well as their chemical composition. The physical and chemical differences are considerably modified by the heredity of the variety and the influence of the climatic conditions in which they are grown. For our purpose, these varieties should be thoroughly studied under the space conditions. The constituents of major interest, protein and oil, make up to approximately 60% of the bean, but about one third consists of carbohydrates including polysaccharides, oligosaccharides (stachyose and raffinose) which are major causes of soybean flatulence, and of sucrose (Wolf and Cowan, 1971; Kinsella, 1979). Proximate composition of soybean and seed parts is presented in Table 8.

Table 8
Proximate Composition of Soybean and Seed Parts
(from Wolf and Cowan, 1971)

Fraction	Protein (N x 6.25) %	Fat %	Carbohydrate %	Ash %
Whole bean	40	21	34	4.9
Cotyledon	45	23	29	5.0
Hull	8.8	1	86	4.3
Hypocotyl	41	11	43	4.4

The bulk of the proteins are stored in the protein bodies which may vary from 2 to 20 μ in diameter. The oil is located in the smaller structures called spherosomes which are interspersed between the protein bodies and are 0.2 to 0.5 μ in diameter (Wolf and Cowan, 1971). Like wheat, before conversion of soybean into its constituent macronutrients, it should initially be cleaned and milled.

Traditionally, industrial practice in almost all soybean processing consists of a series of steps including seed cracking, separation of seed parts, flaking of the predominantly cotyledonous part, hexane extraction of the lipids in the defatting step, desolventizing of the lipid and defatted portions, followed by further fractionation of the lipid and nonlipid portions as desired (Circle and Smith, 1978).

During the past three decades, new processes for producing oilseed protein products have been developed as alternatives to conventional procedures. Several processes have departed radi-

cally from traditional extraction practices in that an aqueous system was employed to extract both oil and proteins (Sugarmann, 1956; Eapen et al., 1966; Bhatia et al., 1966; Rhee et al., 1972; Hagenmaier et al., 1973; Mattil et al., 1979).

In other new and unconventional processes, ultrafiltration membranes have been employed to recover protein from defatted oilseed flour extracts as an alternative to isoelectric precipitation (Lawhon et al., 1977, 1978, 1979, 1980; Nichols and Cheryan, 1981).

A combination of aqueous extraction and membrane filtration has also been employed to produce a variety of oilseed products containing different ratios of protein and oil (Olsen, 1978; Cheryan and Schlesser, 1978; Omosaiye et al., 1978; Omosaiye and Cheryan, 1979; Lawhon et al., 1981A, 1981B).

Although there are some inherent disadvantages in aqueous processing of soybeans (e.g. lower efficiency, reduced product stability, and risk of microbial contamination), there are, however, overriding advantages of utilizing this technique under CELSS conditions. The most important of these would be safety (absence of flammable organic solvents) and lack of solvent pollution of food and environment. These considerations make the aqueous processing of soybeans the method of choice under space conditions.

a. Soy Proteins

Soybean proteins consist of a complex mixture of components with a wide range of molecular weight. Soybean proteins are generally divided into two categories: "globulins" and "whey proteins." The globulins or storage proteins are located in the protein bodies, and precipitate at pH 4.5 to 5.0, which is their isoelectric region (Wolf, 1978). The whey proteins are composed of intracellular enzymes (lipoxygenase, urease, amylase),

hemagglutinins, protein inhibitors and membrane lipoproteins (Wolf, 1978; Kinsella, 1979).

The protein fractions are designated 2, 7, 11, and 15s based on their sedimentation rates. Approximate amounts of the four fractions are given in Table 9.

Table 9

Approximate Distribution of the Major Components of Soy Protein (Wolf and Cowan, 1971; Kinsella, 1979)

Fraction	Percent of Total	Components	M.W.
2s	8	Trypsin inhibitors,	8,000 - 21,500
		Cytochrome C.	12,000
7s	35	Hemagglutinins	110,000
		Lypoxigenases	102,000
		Beta-amylase	61,700
		7s Globulins	180,000 - 210,000
11s	52	11s Globulins	350,000
15s	5	Polymers	600,000

The storage proteins, 7s (conglycinin) and 11s (glycinin), are the principal components of soy protein. The relative quantities of these proteins, according to literature data, vary

widely. the discrepant data may be attributed to the association-dissociation properties of these proteins under different conditions (Kinsella, 1979). The processed soybean proteins, used as food ingredients, are conveniently divided into 3 categories based on protein content:

- Soy flour and grits
- Soy protein concentrate
- Soy protein isolate.

It seems appropriate to focus only on the soy protein isolate. Although for the extrusion process (discussed later), soy meal or soy flour containing much less protein can also be used. This would exclude any considerable interference from other soy minor constitutents which may not be present in other proteinaceous systems.

Soy protein isolates, as the most refined form of soy proteins, are processed one step further than the concentrates by removing the water-insoluble polysaccharides as well as water-soluble sugars and other minor constitutents.

Defatted soy proteins (via aqueous extraction) are extracted with dilute alkali (pH 7 to 9) at 50 to 55°C. The extract is then separated from the insoluble residue (water-insoluble polysaccharides plus residual protein) by screening, filtering, and centrifugation. On adjusting the pH of the extract to about 4.5 (the isoelectric region) with food-grade acid the major protein precipitate. After filtering and/or centrifuging the protein curd from the solubles (whey), it is washed with water. Direct spray drying of the curd yields the "isoelectric protein" while neutralization followed by spray drying gives the "proteinate" form of the isolate (Wolf and Cowan, 1971). Proteinates are usually preferred because they are water-dispersable and therefore easier to incorporate into food products. Figure 2 shows production of soybean protein isolate.

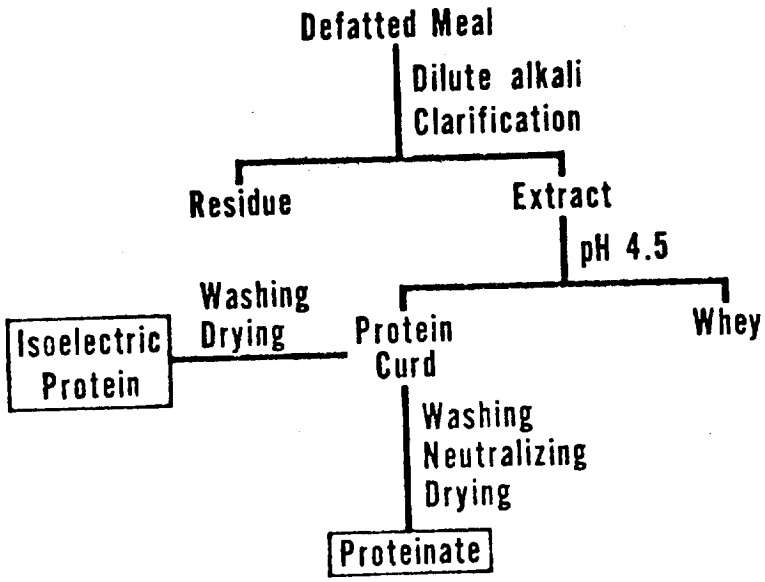

Figure 2. Diagram for production of soybean protein isolates (from Wolf and Cowan, 1971)

Proximate analysis of these two forms of soy isolates is represented in Table 10.

Table 10
Proximate Analysis of Soy Protein Isolates
(from Meyer, 1967)

	Proteinate	Isoelectric Protein
Protein, %	92.2-92.8	92.2-94.7
Moisture, %	4.7-6.4	3.7-7.6
Crude fiber, %	0.1-0.2	0.1-0.2
Ash, %	3.5-3.8	2.0-2.7
Nitrogen Solubility Index	85-95	-
pH (1:10 aqu dispersion)	6.8-7.1	5.2-5.5

The non-protein materials may affect physical and chemical properties of isolates. For example, it has not been demonstrated that the residual lipids and other non-protein constituents contribute to the flavor of isolates, but they are likely suspects for flavor defects arising when isolates are incorporated into certain food products. "Painty" flavors, for example, have been reported for spun fibers prepared from isolates (Kuramoto et al., 1965). Functional properties or physicochemical properties which give information on how a protein will behave in a food system is of particular importance in CELSS. Summary of functional properties of soy proteins important in food applications are listed in Table 11.

Table 11

Summary of Functional Properties of Soy Proteins Important in Food Applications (Kinsella, 1979)

Property	Functional Criteria
Organoleptic/kinesthetic	Color, flavor, odor, texture, mouthfeel, smoothness, grittiness, turbidity
Hydration	Solubility, wettability, water absorption, swelling, thickening, gelling, syneresis
Surface	Emulsification, foaming (aeration, whipping), protein-lipid, film formation, lipid-binding, flavor-binding
Structural/rheological	Elasticity, grittiness, cohesiveness, chewiness, viscosity, adhesion, gelation, dough formation, texturizability
Other	Compatibility with additives, enzymatic antioxidant

Nutritional Aspects of Soy Proteins

Soybean proteins are highly digestible and have an essential amino acid pattern, which is one of the best among vegetable protein sources. For example, the relative high lysine content of soybean protein makes it an effective supplement to wheat protein, which is low in lysine (Bressani, 1981).

Soybean amino acid pattern resembles, with the exception of the sulfur amino acids, the amino acid patterns of high-quality animal protein sources.

Table 12

Essential Amino Acids in Soybean Protein in Comparison with Meat and Milk (mg/g N) (from Bressani, 1981)

Amino Acid	Soybean	Meat	Milk
Isoleucine	336	327	407
Leucine	482	512	626
Lysine	395	546	496
Phenylalanine	309	257	309
Tyrosine	199	212	325
Cystine	111	79	57
Methionine	84	155	156
Threonine	246	276	294
Tryptophan	86	73	90
Valine	328	347	438

Overall supplementation and modification of soy proteins can make this protein source more suitable for human consumption (Watt and Merrill, 1975; Liener, 1977; Young et al., 1979).

Soy protein is deficient in methionine and supplementation

may be necessary. There is also concern about the possible role of soybean proteins in reduction of bioavailability of minerals (especially zinc and iron). However, there is no evidence that soy protein per se directly affects the bioavailability of minerals from soya (Erdman and Forbes, 1981; Torun et al., 1981).

Trypsin and chymotrypsin inhibitors are known to cause adverse physiological reponse in animals, and, unless destroyed (e.g. thermal denaturation via cooking) can detract from the full nutritional potential of soy protein (Rackis, 1981; Liener, 1981; 1976; 1978).

Flavor limits the use of soybeans. The amount of soy that can be incorporated in a particular food is limited by the natural heavy or bitter flavor of soybeans. However, the presence of other ingredients as well as treatments such as baking, roasting, cooking, and extrusion, may help to alleviate the problem of unpleasant flavors (Rackis et al., 1979; Schuttle, 1979).

By using various temperature-time treatments the trypsin inhibitors are inactivated as well as the proteins denaturated and the nutritional value improved (Liener, 1981; Liener, 1976; Liener, 1978; Banks and Pringle, 1976; Ellenrieder et al., 1981; Ellenrieder et al., 1980; Camacho et al., 1981; Van Stratum, 1978). Properly heat treated soy protein materials are not expected to pose any health problems (Van Stratum, 1978).

b. Soy Oil

Oil is the most concentrated form of energy-producing nutrients. Soy oil may be used directly in the consumption area or along with other nutrients in the food fabrication area of CELSS. The major fatty acid composition of soybean oil is as follows (Dugan, 1976): saturated 14.0%, oleic 22.9%, and linoleic 55.2%. Factors such as lack of cholesterol and low

saturated fat are desirable characteristics of soy oil.

Extraction of oil from soybeans is achieved, as described earlier, by aqueous extraction process. Crude oil contains materials that must be removed to provide the minimum desirable characteristics of purity, color, odor, and flavor (Dugan, 1976; Wolf and Cowan, 1971). Under these conditions, soy oil could be regarded as a macronutrient and can be substituted by other plant oils. Under CELSS conditions, the minimum steps for processing of soy oil will be as follows:

1. Settling and degumming. This step is utilized to remove suspended and colloidally dispersed matters such as proteins, carbohydrate residues, water, and phospholipids. Upon storing of heated fat, water and materials associated with water settle into the cone, where they are drawn off. Purification of soybean oil requires removal of phospholipids, usually referred to as "lecithin". Degumming is accomplished by mixing the oil up to 2% water of steam for one-half hour at 54-71°C. The hydrated lecithin is separated from the oil by centrifugation or settling. the obtained lecithin could be used as an "emulsifier" in food fabrication of CELSS.

2. Alkali refining. Refining with alkali removes free fatty acids which can result from lipolysis prior to rendering or extracting the oil. Caustic soda solution (sodium hydroxide) are added to the heated oil and stirred vigorously. After a short period, the mixture is allowed to settle. The settlings can be used as "soap stock". In addition to removing free fatty acids, refining also removes phosphatides, some solid matter, and some of the colored matter mainly by occlusion with the soaps.

3. Bleaching. Bleaching decolorize the oil by adsorbing the colorants on bleaching earth (not recommended for CELSS) and/or activated carbon (prefered for CELSS). In addition to decolorization, bleaching agent may also absorb suspended matter,

soaps, phosphatids, and water. Deaerated and dried oil is mixed with the bleaching agent, the slurry is heated to 105 to 110°C, and sprayed into an evacuated chamber to remove any bonded moisture released by heating the bleaching agent. The oil and bleaching agent are separated in closed filter press.

4. Deodorization. Substances contributing to undesirable flavor and odor must be removed. Deodorization can be done by several different approaches. Basically by the use of low pressures (3-6 torr) and high temperatures (170-230°C) with the passage of steam through the oil, the volatile products are removed from the oil. This steam-vacuum deodorization process removes volatile substances, such as aldehydes and ketones, destroys peroxides and carotenoid pigments, and strips out residual free fatty acids.

The soybean oil treated as above should have a bland taste and clear color. Extra steps such as winterizing and hydrogenation do not seem to be necessary under the CELSS conditions.

C. Macronutrient from Algae

Proposals on algae utilization in space habitats have been based on their multifunctionality. Algae can be used primarily as a photosynthetic gas-exchanger (CO_2 consumption and O_2 generation) with no need for provision of organic carbon substrate. The algae biomass is also potentially a good source of protein (SCP) which can be purified and utilized along with other recovered macronutrients in the space habitat. Other functions of algae include water purification, waste processing, nitrogen fixation, and removal of volatile gases. There are 3 genera of algae (Spirulina, Scenedesmus, and Chlorella) with relatively known nutritional safety records, of which Spirulina has been studied the most.

The nutrient content of algae reflects to a great extent the composition and environmental conditions of the cultivation media. The published results on the chemical composition of "whole" algae (Clement et al., 1967; Lipinsky and Litchfield, 1970; Waslien, 1975; Becker, 1981; and Santillan, 1982) demonstrate these variations. Table 13 compares the chemical composition of 3 algal genera with soybean, while Table 14 compares the nutritive value of algae with egg and milk.

Table 13

Chemical Composition of Different Algae Compared to Soy (% dry matter)(from Becker, 1981)

Component	*Scenedesmus*	*Spirulina*	*Chlorella*	Soy
Crude protein	50-55	55-65	40-45	35-40
Lipids	8-12	2-6	10-15	15-20
Carbohydrates	10-15	10-15	10-15	20-35
Crude fiber	5-12	1-4	5-10	3-5
Ash	8-12	5-12	5-10	4-5
Moisture	5-10	5-10	5-10	7-10

Table 14

Nutritive Value of Algae in Comparison with Animal Proteins (adapted from Chen and Peppler, 1978)

	Digestibility (D)	Biological Value (B.V.)	Net Protein Utilization (NPU = D x BV)
Animal:			
Egg	97	100	97
Milk	97	93	90
Algae:			
Chlorella-Scenedesmus	65	54	35
Spirulina maxima	84	72	60

Direct consumption of single cell biomass without purification is not feasible in the amount which would be of any significance to biomass recycling. This is because of physiological concerns

(due to undesirable components, e.g., excess nucleic acids, toxic pigments and metals, steroids, nondigestive components of cell wall and disturbing carbohydrates, and unknown toxins and allergens) as well as organoleptic concerns (offensive flavors, taste, color and texture).

Since there are unknown numbers of undesirable or potentially undesirable components in the algae system for consumption as food, we in this study focus only on the recovery of the major edible component (i.e. proteins) in relatively pure form.

The overall nutritional and safety considerations of SCP have been recently reviewed by Tuse (1983). However, it seems that there has not been any compositional, toxicological, nutritional, and technological (functional properties) studies on the purified algal proteins. This research is therefore of prime importance and should be carried out before the actual utilization of algae as a protein source in space habitat. To isolate the "safe and nutritious" algal proteins, the first task is cell rupture.

Cell walls represent about 10% of the algal dry weight (soeder, 1978). They consist mainly of complex polysaccharides and murein, the typical structural macromolecules of bacterial cell walls. In Spirulina amino glucosan and amino rhamnosan make up for 11-12% of the dry matter of the cell wall (Soeder, 1978). The intact, complex polysaccharide cell wall is an obstacle to digestibility. The feasibility and application of chemical, enzymatic, and physical methods to break the cell wall of micro algae and other single cell proteins (SCP) have been reported in the literature.

a. Chemical Method: Chemical methods include the use of urea, guanidine, sodium hydroxide (Mitsuda et al., 1969; Huang and Rha, 1971), alkaline biocarbonate buffer (Tannenbaum et al., 1966), acetic acid, oxalic acid, citric acid (Samerjima et al., 1971), methanolic hydrogen chloride (Tamura et al., 1972) and

ethanol acetone (Lee et al., 1979) among others.

b. Enzymatic Method: This method includes incubation of active cells at the optimum temperature to induce the lytic reaction of endogenous enzymes or addition of enzymes to lyze the cell wall (Hedenskog et al., 1969; Maul et al., 1970; Castro et al., 1971; Carenberg and Heden, 1970).

c. Physical Method: These methods involve the rupture of cells with a high-pressur press, freeze-thaw treatments, and sonication, as well as high-speed ball mill grinding, high pressure homogenization, and high-speed mixing (Hedenskog and Mogren, 1973; Lee et al., 1979; Cunningham et al., 1975; Dunnill and Lilly, 1975).

Each of the above methods has its own advantages and disadvantages. For our study, we should bear in mind the special conditions of space habitats. For this purpose we should avoid using organic solvents, and focus on simple chemical treatments (acid, alkali) and on simple mechanical and enzymatic methods.

Upon completion of this stage, we expect to obtain a homogeneous mixture of cell wall fragments, various released cytoplasmic proteins and cell organelles, nucleic acids, and pigments, from which we are interested in separation, concentration, and isolation of the nutritionally acceptable proteins. The utilization of the remaining components could be the subject of additional research activities in the area of by-product management.

Protein extraction is usually attempted concurrently with reduction of nucleic acids. Nucleic acids, insoluble below pH 4.5, if ingested directly, lead to the elevation of blood uric acid levels as a result of their degradation to the purine bases, adenine and guanine, and the in vivo oxidation of these latter. Uric acid with extremely low water solubility cannot be further degraded and is only partially excreted. This leads to gout, to diseases affecting the joints and to the formation of "stones"

in, for example, the kidneys and the bladder (Hudson, 1980). Reduction of the levels of nucleic acids is thus of special importance.

The nucleic acid content for algae is, in general, lower than for yeast. For example, it is reported that Scenedesmus has a nucleic acid content of about 4% (Hedenskog, 1978), and Spirulina about 4.5% (Santillan, 1982). On the other hand, the generally accepted safe level of nucleic acid intake in the human is 2 grams per day (Scrimshaw, 1975), and this would limit the daily algal consumption to about 50 grams per day. Thus, if algal proteins are to be used as a major ingredient in fabricated foods in space habitats, reduction of nucleic acids is an essential step.

There are numerous methods for reduction of nucleic acid content in cell suspensions and in cell homogenate. These methods have been reviewed by Sinskey and Tannenbaum (1975), Litchfield (1977), Chen and Peppler (1978), Hedenskog (1978) and Gierhart and Potter (1978). Cell suspensions have been treated with acid (Peppler, 1970), aqueous ammonia (Ayukawa et al., 1971; Akin and Chao, 1973), heat shock/pancreatic RNase (Castro et al., 1971), heat shock/Na_2HPO_4 solution (Canepa et al., 1972), MeOH/HCl mixtures (Tamura et al., 1972), heat shock (Tannenbaum, 1973), EtOH/HCl mixture (Akin and Chao, 1974) heat shock/pH 5.0-5.5 (Akin, 1974), heat shock/carboxylic anion (Sinskey and Tannenbaum, 1975),and NaOH or aqueous ammonia (Viikari and Linko, 1977).

Reduction of nucleic acids in cell homogenates have been reported with NaCl 3%/50°C - pH 5.6 (Lindblom and Morgan, 1974), pH 6.0/80°C (Vannanuvat and Kinsella, 1975), high temperature-low alkali, or low temperature-high alkali (Newell et al., 1975), 100°C/pH 6-8 (Robbins, 1976), extracellular RNase (Fazakerley, 1976), and succinylation after cell disruption (Shetty and Kinsella, 1979). Considering the overall conditions of space

habitats, we should focus on the concurrent protein extraction and nucleic acid reduction (Hedenskog, 1978) which is the most efficient and suitable for our objectives.

Upon extraction of cell contents the protein components are selectively precipitated via their isoelectric properties, at various ionic strengths, in combination with appropriate physical techniques, especially density gradient centrifugation. the pellet may be washed 2-3 times and the process repeated. Attempts should be made to minimize protein denaturation during the extraction and isolation processes since particular characteristics of algal proteins that should be taken advantage of could be lost during the above processes. We also should be cautious about the coprecipitation of nucleic acids at pH 4.3-4.5. This will leave most of the undesirable components (e.g. cell walls, nucleic acids, and pigments) in supernatant or separate phases.

Figure 3 summarizes proposed processes for isolation of algal proteins.

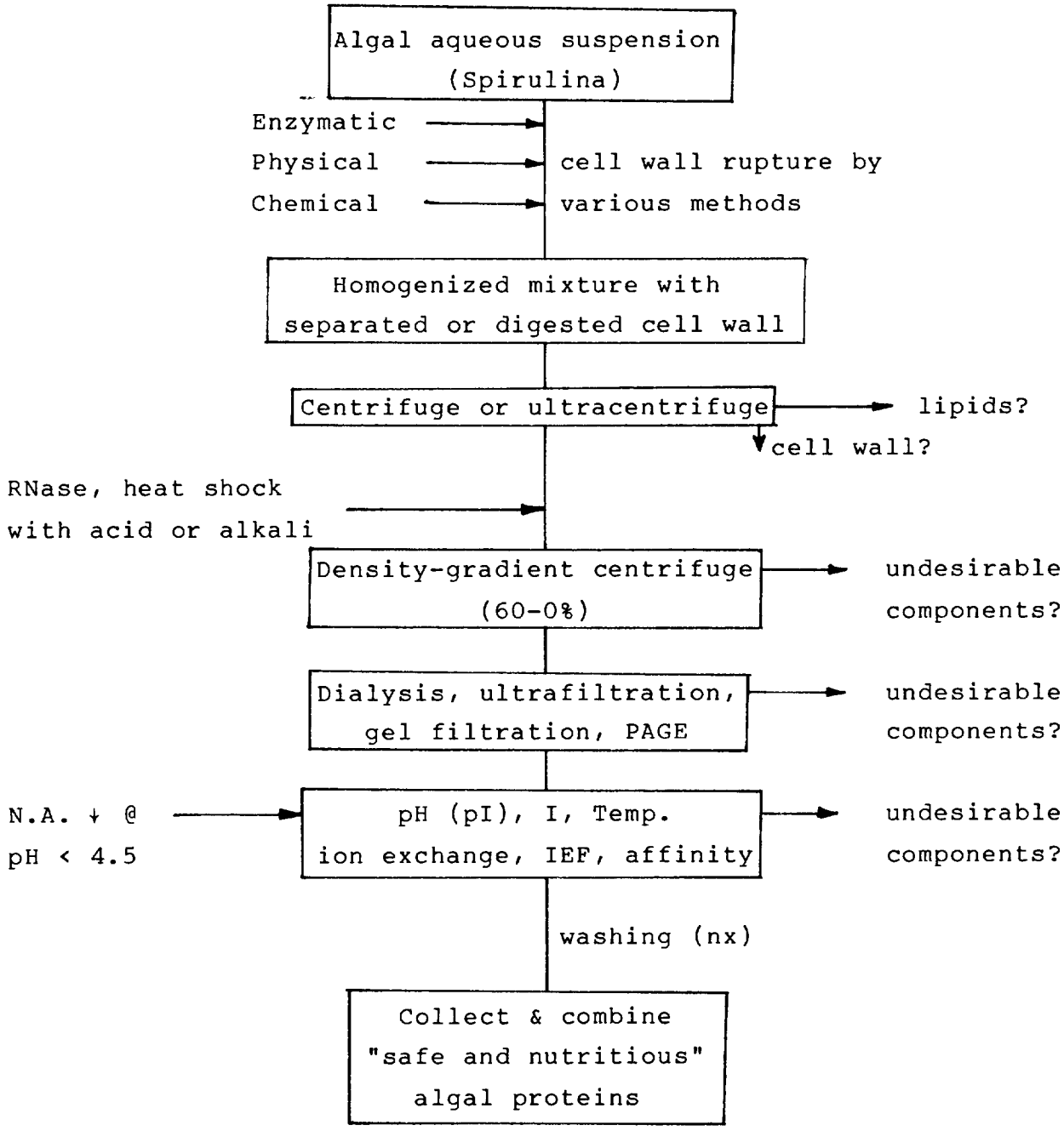

Figure 3. Proposed process for isolation of algal proteins.

D. Glycerol as a Macronutrient

Glycerol is potentially a nutritionally, as well functionally (plasticizing effect) useful ingredient of fabricated foods. It has been the subject of several books and reviews (Miner and Dalton, 1953; Newman, 1968; Lin, 1977; Ashworth, 1979). Glycerol is metabolized in the body to D-glucose, and yields 4 Kcal/g, which is the same as the energy content of other carbohydrates.

Nutritional studies on feeding glycerols to humans were carried out by Johnson et al. (1933). They gave 14 graduate students 30 ml of 95% glycerol (mixed with orange juice to disguise the sweetness) after each of three daily meals for 50 days and observed no ill effects. Since that report, numerous tests have been conducted, and an oral dose of 1 gram of the compound per kilogram of body weight, once every 6 hours is now considered safe (Deichmann, 1940; Tourtellotte et al., 1972). The adult level of serum glycerol is normally 0.05 and 0.1 mM (Lin, 1977). Fairfull-Smith et al. (1982) found that 3% amino acids plus 3% glycerol are safe in solution to give by peripheral vein to post-surgery patients for 5 days. The addition of glycerol improves nitrogen balance as compared with amino acids alone. Under properly controlled conditions, 50 g of glycerol in a 5% solution can be administered by intravenous injection to human subjects without adverse symptoms (Sloviter, 1958). The proportion of glycerol excreted will depend on the nutritional status of the recipient, the amount of food taken, and on the dose ingested. Newman (1968) quotes the figures given as follows.

Relation between Ingestion and Excretion of Glycerol
(Newman, 1968)

Amount Ingested	Amount Excreted
20-30 grams	0-1%
20-50 "	1-3%
50-100 "	3-10%
100-150 "	10-15%
150-200 "	15-28%

These figures are probably applicable to an adult of average weight. Most of the glycerol was excreted in urine, with negligible amounts in the feces.

Regarding the effect of large, single doses on humans, Ferber and Rabinowitch (1929) noticed no untoward reaction on offering 100 grams of glycerol diluted to 300 ml, and a similar result was reported by Gissel (1933) involving a dose of 200 grams. Latven and Molitor (1939) developed data which indicate that dose of up to 480 g of glycerol (which is many times the level one would be likely to allocate to this energy source) are not toxic. A more serious problem at lower levels of acceptability is the organoleptic acceptability. Shapira, (1970A) in a ninety-day study of glycerol as a human diet supplement, observed no nausea or ill-effects, although there was a rise in the subjects' urine-free glycerol content.

The amount of glycerol which is tolerable depends on several factors, including in particular the other components of a meal, and if glycerol is to be used in space this aspect will require further study. Industrially, glycerol is produced from natural fats and oils, from catalytic cracking and hydrogenation and also by fermentation. None of these methods seem applicable for the

space station. The formation from petroleum products and from fats presumes the availability of these raw materials, and if yeast production were to be considered, other end production would be of equal, or greater interest than glycerol.

There are also a number of chemical methods for synthesis of glycerol. Formation of glycerol by chemical means has the advantage of freeing the production of this food energy source from the dependence on land and/or unexpected production interruptions. Three-carbon compounds can be synthesized which could be starting material for glycerol (Pictet and Barbier, 1921). To what extent such synthesis could be carried out in CELSS is not at present known. However, there has been some work on the synthesis of nutrients from simple chemicals, and some of this work was directed specifically to space conditions (Frankenfeld, 1967). It is known that a number of 3-carbon compounds such as glyceraldehyde, dihydroxyacetone, allyl alcohol, acrolein can be used as the starting material for glycerol synthesis (Newman, 1968).

One approach which has been investigated by NASA as well as other investigators is the conversion of formaldehyde to "formose" sugars. Hydrogenolysis of "formose" produces glycerol, 1,3-propanediol, and 1,4-butanediol which are believed to be at least partially utilizable as energy sources and which can be produced also by other synthetic routes (Shapira, 1968; Shapira, 1970B; Weiss and Shapira, 1971). The formaldehyde is assumed to be attainable with suitable energy input by catalytic conversion, from CO_2 and H_2O as follows:

$$4H_2O \rightarrow 4H_2 + 2O_2$$
$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$
$$O_2 + CH_4 \rightarrow HCHO + H_2O$$

Homogeneous $Ca(OH)_2$ catalyzed condensation of formaldehyde (HCHO) to glycolaldehyde initiates the formose reaction. Once glycoaldehyde is formed, the reaction behaves as the well-known base-catalyzed aldol condesnation, and subsequent formaldehyde addition at the α-hydrogen proceeds readily to form glyceraldehyde. Glyceraldehyde can then undergo rearrangement to dihydroxyacetone. Formaldehyde addition to the α-hydrogen of glyceraldehyde forms a branched chain tetrose, to those of dihydroxyacetone a straight chain keto-tetrose which then isomerizes to the aldose form. Build-up to higher molecular weight sugars proceeds analogously.

Because the formose sugars are toxic, the interest in the present project lies in production of dihydroxyacetone, and glyceraldehyde which are readily reduced to glycerol (Weiss and Shapira, 1971).

Recently, researchers at MIT have accomplished the total synthesis of all 8 L-hexoses, using a reiterative two-carbon extension cycle consisting of four steps (Ko et al., 1983). Should such procedures produce D-hexoses and prove amenable to utilization under CELSS conditions, they may offer a new approach to synthesis of nutrients.

While we are not considering here the synthesis of triglycerides, it seems appropriate, at this point, that some methods for their production (ACS, 1980) be mentioned for possible future applications. Esterificaion of fatty acids with glycerol presents no problem.

a. The simplest method for synthesizing fatty acid precursor is to separate the paraffin or "straight chain" hydrocarbons from petroleum which can then be converted to fatty acids through oxidation and purification (this method is out of the scope of the present study).

b. The Fischer-Tropsch reaction affords an alternate route to fatty acid precursors. This reaction can convert carbon

monoxide and hydrogen to straight-chained paraffins, which can then be converted to an edible product. The Germans actually employed this process during World War II to produce margarine. The synthetic product varied considerably in quality, but was apparently nontoxic and somewhat nutritious. Howevr, the low overall yield of edible material makes the process unattractive. Still, the Fischer-Tropsch synthesis employs readily available starting materials (carbon dioxide and methane). This technology, old and exhaustively studied (Frankenfeld, 1967; Anderson, 1954, 1955) is currently experiencing a resurgence of interest. New developments should be watched for clues in learning how to modify the synthesis to produce more straight chain hydrocarbons.

c. The third method for synthesis of fatty acid precursor involves the conversion of CO to ethylene $CH_2 = CH_2$ followed by controlled polymerization by means of the reaction discovered by Ziegler in Germany in 1959. This approach produces long-chain olefins (open-chain unsaturated hydrocarbons containing at least one double-bonded carbon) of the right size with virtually no branched chain or other by-products (Frankenfeld, 1967; Ziegler, 1965).

E. Glucose from Cellulose: Saccharification

One assumed route to glucose production in CELSS is conversion of waste cellulose. Glucose may then be used as the natural sweetener with an acceptable taste providing most of the 15% recommended daily energy intake from sugars (III-3), or as a precursor.

Cellulose saccharification has been the subject of many books and reviews (Gaden et al., 1976; Goldstein, 1981; Phillips and Humphrey, 1983). The raw byproducts of CELSS which should finally be converted to glucose are derived from plant production (e.g., wheat and soy straw) as well as processing (e.g., wheat bran and soy hull). Wheat bran may be used for production of cellulases (discussed later). All of the above byproducts have cell walls that define the morphology of the plant, provide its structural support, and control the passage of water and nutrients. The formation, organization and properties of these cell walls are complex (Preston, 1974). Plant cell walls contain skeletal polysaccharides, hemicelluloses, polyuronides, lignin, and proteins. Cellulose (the most important skeletal polysaccharide), the hemicelluloses, and lignin are the chief components of woody plant cell walls. Variation in composition of some CELSS byproducts is presented in Table 15.

Table 15

Approximate composition (% dry wt.) of some CELSS byproducts for cellulose saccharification
(Sloneker, 1976 and Sanella and Whistler, 1962)

Residue	Cellulose	Hemicelluloses	Lignin	Protein
Wheat straw	40	29	13.6	3.6
Soybean straw	41.4	19		5.5
Soybean hull	49.8	16.6	7.8	13.6

a. Cell Wall Components and Organization

Cellulose: Cellulose is a long chain polymer of β-D glucose in the pyranose form, linked together by 1,4-glycosidic bonds to form cellobiose residues that are repeating units in the cellulose chain.

The β-linkage requires that the alternate glucose units must be rotated through 180°. An important implication of this structure is a marked tendency for the individual cellulose chains to come together to form bundles of crystalline order held together by hydrogen bonds. The conformation of the pyranose rings is such that the total energy lies close to the minimum, a major factor in the high stability of cellulose.

Despite the presence of three hydroxyl groups on each anhydroglucose residue in the cellose chain, cellulose is completely insoluble in water. The unnumerable hydrogen bonds holding the chains together are not broken by water. Strong acids or alkalis, concentrated salt solutions, and various complexing

reagents can swell and disperse, or even dissolve the cellulose, breaking up the highly ordered crystallites. On regeneration from dispersion or solution the celluose assumes a different crystalline structure. The degree of crystallinity of celluloses varies with their origin and treatment.

The chain length of cellulose as determined by measurements on cellulose solutions of different origins and treatments shows considerable variations. Values for the degree of polymerization (DP) range from 7,000 to 15,000 (Goldstein, 1981).

Hemicelluloses: Closely associated with the skeletal polysaccharide cellulose in the cell wall are other structural polysaccharides collectively call hemicelluloses (Schuerch, 1963). Hemicelluloses differ from cellulose in that although water insoluble, they can be dissolved in strong alkali. This property may be used to separate the hemicelluloses from the total carbohydrate fraction called holocellulose, leaving essentially pure or α-cellulose behind. Hemicelluloses are also more readily hydrolyzed by acid than is cellulose.

The hemicelluloses consist, for the most part, of sugars other than glucose, both pentoses and hexoses, and are usually branched with degrees of polymerization ranging from less than 100 to about 200 sugar units. Their greater solubility and susceptibility to hydrolysis than cellulose result from their amorphous structures and low molecular weights. Arabinoglycuronoxylans, containing both arabinose and uronic acids, appear in wheat straw, softwoods, and in food crops. Arabinogalactans are water-soluble polysaccharides that are highly branched.

Lignin: The third major cell wall component in woody plants is lignin. In fact, lignin is the necessary component for a plant to be classified as woody. Lignin serves as a cement between the wood fibers, as a stiffening agent within the fibers, and as a barrier to enzymatic degradation of the cell wall. The

occurrence, formation, structure, and reactions of lignin have been treated in detail (Sarkanen and Ludwig, 1971).

The molecular architecture of the plant call wall has a great influence on the utilization of the components.

The cell wall components individually described above are intimately associated in their native state, and, as a consequence, their properties and response to processing are influenced by the close proximity of the other components as well as possible chemical combination with them.

Cellulose occurs in the cell wall in microfibrils that possess a crystalline core surrounded by an amorphouse region. There is some evidence that the basic cellulose structure is an elementary fibril 35 Å wide (four unit crystallite cells) with the microfibril an assembly of four crystalline elementary fibrils. The length of the microfibrils has not been determined, but if a degree of polymerization for cellulose of 10,000 is accepted, the minimum length would be 5000 x 10 Å (the approximate length of the cellobiose residue) or 5 μm. Greater actual values would be expected because of chain overlap, and a continuous fibril is conceivable.

Microfibril widths vary from 100 to 300 Å depending on such factors as aggregation of single microfibrils to form larger assemblies and the amount of amorphous matrix material surrounding the crystalline areas. Hemicelluloses and lignin are the matrix polymers that surround the microfibrils as well as their constituent elementary fibrils. In addition, amorphous regions of the cellulose may also be penetrated by matrix polymers. The cell wall is then a fiber-reinforced plastic with cellulose fibers embedded in an amorphous matrix of hemicelluloses and lignin.

The separation of the components prior to conversion to chemicals is difficult and expensive, and the application of conversion processes to the mixed components could be inhibited, as for

example, the effect of lignin in preventing the access of cellulase to the cellulose.

b. Pretreatments

In order to utilize cellulose, several limitations must be overcome. There are two physical barriers in plant byproducts for cellulose hydrolysis: cellulose crystallinity and presence of lignin. There has been considerable research effort in this area which has been reviewed by Cowling (1975), Millett et al. (1976), Hawlliwell (1978), and Phillips and Humphrey (1983). Applicability of each method or their combination for use under space conditions needs further research in this area.

1. Physical Methods. The principal physical pretreatments are milling and radiation treatment. Milling - ball milling, two-roll or compression milling, hammer milling, colloid milling, cryomilling, disc refining, and so on - decreases the particle size, crystallinity and degree of polymerization, and increases the surface area and the bulk density of the raw material (Phillips and Humphrey, 1983). Vibratory ball milling is more effective, since it not only exposes carbohydrate surface to the enzymes but decreases crystallinity as well. The rate of wood dilute acid hydrolysis was increased fivefold and the maximum yield of sugar obtainable under batch hydrolysis was increased 60 to 140% by ball milling (Millett et al., 1978).

Another milling technique effective in increasing the susceptibility of cellulosic material to enzymatic hydrolysis is differential speed two roll milling (Tassinari and Macy, 1977). It is believed that both decrystallization and depolymerization of the cellulose occur during the milling by analogy with experience with rubber.

Other physical treatments of cellulosic materials to

increase their reactivity include exposure to both high and low temperatures and to high pressure. Ionizing radiation with doses between 5-100 Mrad have been shown to enhance the rate of subsequent hydrolysis with dilute sulfuric acid (Brenner et al., 1977).

2. Chemical Methods. Chemical pretreatments with agents such as NaOH, NH_3, amines, H_2SO_4, HCl, SO_2, hypochlorite, ammonium bisulfite, steam, and polyhydroxy alcohols are more effective than physical pretreatments in catalyzing simultaneously the disruption of the crystalline cellulose structure and removal of lignin (Phillips and Humphrey, 1983). Swelling of cellulose causes significant increases of the hydrolysis rate. Cellulose treated with sodium hydroxide and/or liquid ammonia are known to be hydrolyzed by acid at a faster rate than the original cellulose (Tarkow and Feist, 1969).

The main consequence of the alkaline swelling treatments has been postulated to be saponification of intermolecular ester bonds, thus allowing the wood to swell to a greater extent and permitting increased enzymatic access (Tarkow and Feist, 1969).

Data for the alkaline pretreatment of wheat straw, illustrating the loss of lignin and carbohydrate from and enzymatic susceptibility of the pretreated residue, are summarized in Table 16.

Table 16

NaOH Pretreatment of Wheat Straw
(from Detroy et al., 1981)

Treatment Conditions	Percent Loss			Percent Conversion to Glucose*
	Hemicellulose	Cellulose	Lignin	
3% NaOH (based on straw), 25°C, 1 hr	32	13	17	51
3% NaOH (based on straw), 90°C, 1 hr	60	9	35	75

*10 IU cellulase/gm wheat straw, 45°C, 6 hr.

Since lignin is a major barrier not only to direct access of enzymes to the cellulose, but also to the extensive decrystallization of cellulose by swelling agents, it would appear that delignification should provide a ready solution to the problem of barrier removal. This is a technically successful solution as shown by the high yields obtained from chemical wood pulps in enzymatic hydrolysis (Mandels et al., 1978).

Steaming can improve the digestibility of wood presumably by the comparable hydrolytic effect of acetic acid liberated by the cleavage of acetyl groups in the wood. Steaming of various lignocellulosic materials such as straw and wood at high pressure followed by sudden decompression to atmospheric pressure has been

recently reported to yield a high degree of cellulose digestibility (Iotech Corp., 1978).

Dale and Moreira (1982) have developed a novel pretreatment to increase the rate and extent of cellulose hydrolysis. This technique is called the freeze-explosion method and relies on treatment of the cellulosic material with a volatile liquid (e.g. anhydrous ammonia) under pressure followed by pressure release to evaporate the liquid and reduce the temperature. Volatile liquids which also chemically swell and decrystallize cellulose are particularly effective when used in this method. Greater than 90% of theoretical conversion of cellulose to glucose has been achieved by enzymatic hydrolysis of alfalfa and rice straw with this technique.

A final approach to chemical pretreatment to enhance cellulose hydrolysis is the selective dissolution of the cellulose from the lignocellulosic raw material, followed by its precipitation in amorphous, highly reactive form. Solvents which have been found to be effective in simultaneously removing the cellulose from the lignin and decrystallizing it include cadoxen (tris [ethylenediamine] cadmium hydroxide), CMCS (aqueous solution of sodium tartrate, ferric chloride, sodium hydroxide, and sodium sulfite), and concentrated sulfuric acid (Ladisch et al., 1978; Tsao et al., 1978).

c. Saccharification

There are two approaches for converting a biomass component to desirable product (Goldstein, 1981): Simultaneous conversion of all its components, or reactions which more or less selectively convert one of the components (e.g. cellulose) at a time to useful products (e.g., glucose) or intermediates. In some cases such reactions may be selective enough that the components other than the target one are virtually unaffected, while in

other cases it might be necessary to bring about at least partial separation of the biomass components before reaction. Preliminary separation may also be necessary where a biomass component interferes with the desired reaction, for example lignin with enzymatic hydrolysis of cellulose. In the following section only, the selective conversion of cellulose to glucose is considered.

Since the reactions such as hydrolysis are also capable of converting the hemicellulose components of the biomass to sugars which would afford an undesirable mixture of products, it is expedient to first remove the hemicelluloses by prehydrolysis before conversion of the cellulose.

The structure of cellulose as a linear polymer of glucose units provides a conceptually simple mechanism for its chemical utilization, namely depolymerization to glucose. Hydrolysis to glucose can be effected by either acid or enzymes, but in neither case is it as facile as the hydrolysis of starch. These hydrolysis reactions as well as the barriers to hydrolysis resulting from the highly ordered crystalline structure of cellulose or the presence of lignin are discussed below.

1. Acid Hydrolysis.

Acid hydrolysis has been developed along two paths. These are dilute acid hydrolysis at elevated temperatures and concentrated acid hydrolysis at lower temperatures.

Dilute acid hydrolysis. Dilute acid hydrolysis is conducted at acid concentrations of less than 2%, at temperatures between 160°C and 250°C, and at reaction times of less than 1 h (Phillips and Humphrey, 1983). Extreme resistance of cellulose to hydrolysis is not attributable to the high polymeric nature of cellulose since starch does not present the same difficulties. It narrows down to the β-glycosidic linkages and the resultant crystalline structure of cellulose. The slow hydrolysis permits

the secondary degradation of the glucose produced to be significant. The reaction of cellulose with dilute acid is a four-step sequence, consisting of (1) a rapid conversion of native cellulose to a stable hydrocellulose fraction, (2) a first-order cleavage of the hydrocellulose to soluble polysaccharides, (3) a rapid hydrolysis of the soluble polysaccharides to simple sugars, and (4) a first-order decomposition of the sugar to a degraded product. Since both the conversion of native cellulose to hydrocellulose and the hydrolysis of the soluble polysaccharides to simple sugars are rapid, this sequence has been simplified to two first-order reactions consisting of hydrolysis of the cellulose to simple sugars followed by degradation of the sugars (Wenzl, 1970).

Kinetic equations describing cellulose hydrolysis were developed by Phillips and Humphrey (1983):

$$C_x \xrightarrow[k_1]{} C_1 \xrightarrow[k_2]{} C_0$$

where C_x = native cellulose
C_1 = simple sugars
C_0 = degradation products.

All reactions were considered first order, and obey the Arrhenius equation. Phillips and Humphrey (1983) reported constants for woods, paper and Barley straw.

Degradation reactions of the glucose in acidic solution result in many products, most of which are highly reactive. Because they are present in only very low concentrations they are difficult to isolate. Thus the kinetics are not simple first order and the mechanism for glucose degradation is complex. Low

yields of hydroxymethylfurfural (10 to 20%) and higher yields of levulinic acid (30 to 50%) have been obtained from glucose in dilute mineral acid solutions (Harris, 1975).

Percolation processes are based on the rapid removal from the reaction zone of the sugars formed during hydrolysis to minimize their further decomposition. The most recent modification of the percolation process separates each cycle into two phases, a reaction step and a washing step. During the reaction step the cellulose is hydrolyzed to sugar under stringent conditions, while in the wash step the sugars formed are dissolved away from the wood under milder conditions. With this scheme, it is possible to obtain sugar solutions of 9 to 10% concentration which can be quite sharply separated into a xylose fraction and a glucose fraction based on the different composition of the wash liquors as the hydrolysis progresses. A mixed fraction is almost completely avoided (Eickemeyer and Hennecke, 1967).

Dilute acid hydrolysis of cellulose to glucose at elevated temperatures is feasible on a commercial scale and capable of providing sugar solutions of 10 to 12% concentration in 50% yield. Lignin does not interfere with the hydrolysis (Goldstein, 1981). The maximum yield of glucose attainable by this method, however, is limited as a practical matter to about 50% from cellulose with a crystallinity as high as that in wood. Although higher yields are possible for very short reaction times at higher temperatures, heat transfer requirements for both heating to the reaction temperature and quenching will limit the extent to which the reaction time can be shortened.

Cellulose with lower crystallinity, either in its native state or as a result of prior processing or pretreatment, is more amenable to hydrolysis and could provide higher yields.

Concentrated Acid Hydrolysis. Concentrated acid hydrolysis is conducted at more moderate temperatures (20-100°C), at high

acid concentrations (>35% HCl; >60% H_2SO_4), and for times varying between 10 min and 6 h (Phillips and Humphrey, 1983). The yield limitations inherent in the high temperature dilute acid hydrolysis of crystalline cellulose are not present in strong acid hydrolysis at low temperatures. Although technical activity and commercial application has been greater for the dilute acid processes, more recently, improvements in corrosion resistant materials and acid recovery techniques have given renewed inpetus to strong acid hydrolysis (especially hydrochloric and sulfuric acids).

The basic principle on which strong acid hydrolysis processes depend is the disruption of the crystalline structure of the cellulose by solution or swelling in the acid. Subsequent hydrolysis of the cellulose can then be accomplished at temperatures low enough to avoid degradation, thereby affording almost quantitative yields of glucose.

After dissolution, the first step is the degradation of the cellulose to oligosaccharides which are then further hydrolyzed to glucose at rates which vary with acid concentration and temperature. Hydrolysis kinetics may vary significantly with only small changes in acid concentration and temperature. In 16 hrs at room temperature, 41% hydrochloric acid caused 100% hydrolysis, 40% HCl 73%, 39% HCl 45%, and 38% HCl caused only 22% hydrolysis.

Only minor degradation of cellulose occurs in 4 hr with 35% hydrochloric acid. Increasing the acid concentration to 40% gives quantitative hydrolysis in 4 hr at 40°C and in only 10 min at 60°C. Up to about 30°C the cellulose is only degraded to oligosaccharides. It is only at higher temperatures that the oligosaccharides are hydrolyzed to glucose by a first-order reaction whose velocity constant increases rapidly with temperature (Lebedev and Bannikova, 1961).

For sulfuric acid solutions, the swelling and solubility of

cellulose at 20°C increase until a maximum at 62% sulfuric acid is reached. Over the temperature range 20 to 40°C the swelling and solubility maxima occur at about 62 to 63% acid. At higher or lower temperatures, the swelling maximum decreases (Vyrodova and Sharkov, 1964).

Solubility and hydrolyzability of cellulose in sulfuric acid do not always follow the same trend, with the presence of water and ethanol often increasing solubility but not hydrolysis. The addition of glucose to the acid lowers the swelling. This effect can influence the hydrolysis when reaction products complex with the acid unless the original and acid-cellulose ratio is high enough to compensate. It appears that the effective concentration of sulfuric acid in the hydrolysis of cellulose must remain above 60% (Sakai, 1965).

It has been found that the acid concentration decreases steadily during hydrolysis with both hydrochloric and sulfuric acids, and that the reaction comes to an end with the establishment of an equilibrium caused by the complexing of the acid with the sugars formed during the hydrolysis (Chalov et al., 1966). Here again the acid to cellulose ratio must be large enough to allow the reaction to proceed to completion.

Strong acid hydrolysis of cellulose is capable of providing yields of crystalline glucose of 85% and even higher glucose yields in solutions suitable for fermentation. These improved yields over dilute acid hydrolysis are offset by higher demand for corrosion resistant equipment and operations associated with acid recovery and loss.

2. Enzymatic Hydrolysis

The alternative to acid hydrolysis is enzymatic hydrolysis which is a complex phenomenon. The specificity of the enzymatic reaction eliminates the problem of sugar degradation. However, enzymatic hydrolysis proceeds at a significantly slower rate than

acid hydrolysis; the size of the enzymes that catalyze the hydrolysis of cellulose relative to the size of the probes within the substrate restricts their access to the reaction sites. Production of enzymes and saccharification of cellulose are two major steps of enzymatic hydrolysis.

The complex of enzymes that hydrolyzes the β-1,4-glucan linkages of cellulose is referred to as cellulase. In general, these enzymes are classified as β-1,4-glucan-glucanohydrolases. If the product of the attack by the enzyme is glucose, the enzyme is specifically designated as a β-1,4-glucan-glucohydrolase. If, however, the product is cellobiose, the enzyme is classified as a β-1,4-glucan-cellobiohydrolase. Those cellulases that attack the substrate in a random manner are given the additional designation of endo-glucanases; those that cleave glucoside units from the terminal end of the polymer chain are denoted as exo-glucanases. This rather systematic classification is often confused by the use of generic terms such as AVICELase, filter paperase, and CMCase, which reflect the activity of an enzyme against a particular substrate rather than the inherent nature of the reaction catalyzed by the enzyme.

The exact nature and mode of action of the components of the cellulase system are matters of continuing research and controversy. Reese et al. (1950) initially suggested that the cellulase system contains a component, C_1, which allows digestion of higher ordered forms of cellulose as well as C_x enzyme components capable of acting on easily accessible forms of cellulose and finally β-glucosidase converts cellobiose and cellotriose to glucose.

The purified C_x fractions have subsequently been designated as endo-β-1,4-glucanases and exo-β-1,4-glucanases. The endo-glucanases, the randomly acting C_x, components, hydrolyze high-molecular-weight glucans; the major products of the reaction are cellobiose and cellotriose. The rate of hydrolysis of the glu-

cans by these endo-glucanase components increases with the degree of polymerization (DP), within the limits of substrate solubility. Endo-glucanase action is associated with a rapid change in solution fluidity and a relatively slow increase in reducing end group production. The exo-glucanases, the endwise acting C_x components, successively remove the glucose from the nonreducing end of the glucan polymer, resulting in a rapid increase in reducing end groups.

A true understanding of the function of C_1 enzyme has been clouded by its association with C_x components. From data on purified C_1 preparations from which the C_x contaminant has been removed, the C_1 component has been identified as an exo-glucanase.

In its purest form, the enzyme is incapable of solubilizing native cellulose. Cellobiose is the major product when the C_1 enzyme is contacted with swollen cellulose for an extended period. Consequently, C_1 is now understood to be a β-1,4-glucan-cellobiohydrolase. It is not solely responsible for initiating the attack on crystalline cellulose; the accompanying action of the C_x glucanases is required to accomplish this hydrolysis effectively. Cellulose hydrolysis is thus presently thought to be a sequential attack in which the randomly acting C_x glucanases initiate the attack and the new chain ends that are produced are then hydrolyzed instantly by the C_1 component in order to prevent reformation of the glycosidic linkage.

In addition to the endo-glucanase and exo-glucanase activities, the cellulase systems of most organisms include a β-glucosidase, sometimes referred to as cellobiase. The primary action of this enzyme is the hydrolysis of cellobiose. In most cases, this enzyme has been shown to have activity against higher oligosaccharides, but the activity decreases markedly as the degree of polymerization increases. In addition, β-glucosidase is less specific about the nature of the glucan linkage it will

attack; it will hydrolyze β-1,1, β-1,2, β1,3, and β-1,6 bonds.

The generalized scheme of multiple-enzyme action of the cellulase system is summarized in Figure 4.

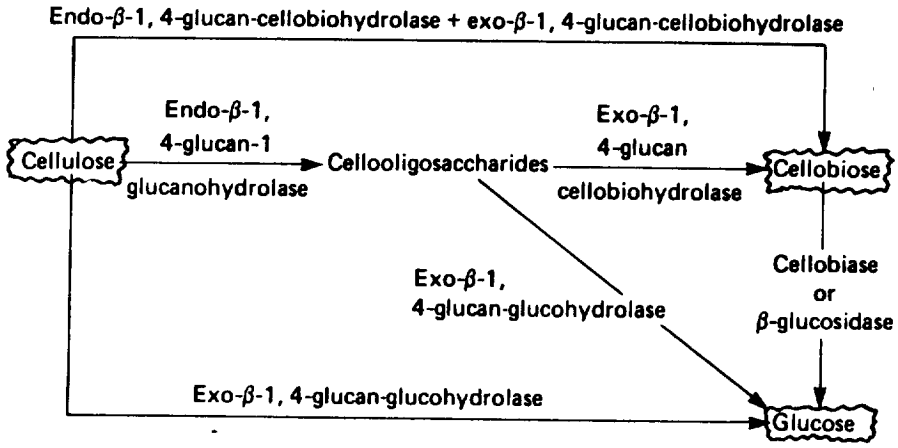

Figure 4. Generalized mechanism of enzymatic cellulose hydrolysis (from Phillips and Humphrey, 1983).

Among the fungi which have been studied for their ability to degrade cellulose and those which have been found to produce cell-free enzymes that can be used to hydrolyze cellulose, one species, Trichoderma viride, stands out for the ability of its extracellular enzymes to hydrolyze crystalline cellulose and for the storage stability of these enzyme preparations. Goldstein (1981) reported that in the Koji semisolid fermentation process used in Japan for production of T. viride cellulase, wheat bran is piled in trays and inoculated after steaming. A water mist spray and forced air circulation during growth are accomplished by frequent turning to ensure good aeration. The enzyme is precipitated from the extract after filtration. Of the many microbial sources of cellulolytic enzymes, the fungus T. reesei has been the most extensively researched (Phillips and Humphrey,

1983).

Enzyme production by fungi is usually adaptive; the enzyme complex is induced or enhanced by the presence of the substrate. Cellulose itself, however, is not the inducer; low levels of the soluble sugars resulting from cellulose hydrolysis activate cellulase synthesis. The cellulases of fungi are usually secreted, from living cells, into the culture medium.

Mutant strains of organisms, selected for rapid production of high concentrations of cellulase and insensitive to catabolite repression, will be of major importance in future progress (Mandels et al., 1978). Enzymatic hydrolysis, while promising, presents many obstacles for large scale operations. The factors that influence the rate and extent of cellulose hydrolysis include the enzyme concentration, the susceptibility of the substrate to hydrolysis as determined by its degree of crystallinity and lignin content, inhibition of the cellulase components by the hydrolysis products, and thermal inactivation of the enzymes during the reaction.

In combination the above factors lead to a rapid decrease in hydrolysis rate with time. After initial rapid hydrolysis of amorphous cellulose, the residue becomes increasingly crystalline. Inhibition by reaction products and enzyme inactivation are both more severe for the cellulase components which act on crystalline cellulose so hydrolysis of the crystalline residue becomes even slower. Methods of the enzyme application (e.g. immobilized) and percent glucose recovery after hydrolysis, would be very important under space conditions and deserves a thorough study. The best pilot scale production of sugar reported to date has been a 6.5% solution in 4 hrs from 26% yield or a 10% solution in 25 hrs for 40% yield using enzymes from Natick strain QM9414 at 45°C in a 100-l reactor (Mandels et al., 1978).

d. Products from Glucose

Although the main purpose of cellulose saccharification is production of glucose as sweetener and energy source in CELSS, the opportunities for further conversion of glucose to useful organic chemicals are however multiple and varied. Ethanol, acetic acid, lactic acid, sorbitol and ascorbic acid are among the chemicals which could be produced and used in the CELSS food system. Phillips and Humphrey (1983) have reviewed the great range of chemicals and food ingredients obtainable from glucose.

VI. FABRICATION OF "FOODS"

As indicated under objectives, our final objective is to convert the macronutrients produced in the space habitat as well as those which are initially suplied from earth into safe, nutritious, and acceptable "food". This process is called "food fabrication" and is presented in Fig. 1.

The need for "foods" rather than "nutrients" is probably best explained by Pyke (1968) in his book, "Food and Society," as follows: "A living cell, or the great commonwealth of cells which together make up a human body, requires for its nourishment the list of proteins -- or, rather, the selected amino acids of which the proteins are composed -- and the fatty substances, the mineral salts, and all the rest which are set down in the biochemical literature and tabulated in the nutritional recommendations of official commissioners. This is what the human body requires. But the human being to whom the body belongs does not necessarily see things in the same light. The fact remains that men and women living in the societies which make up the human population do not ingest nutrients, they consume foods. More than this, they eat meals. Although to the single-minded biochemist or physiologist, this aspect of human behavior may appear to be irrelevant or even frivolous, it is nevertheless a deeply ingrained part of the human situation which exerts a very direct and profound effect on nutritional status and health." In essence, "people eat food, they do not ingest nutrients."

A. General Aspects

There is a tremendous volume of literature and patents on food fabrication. Fabricated foods have been reviewed, among others, by Glicksman (1971), Gutcho (1973), Inglett (1975), and Kinsella (1978).

a. Definition

Definition of foods as "fabricated" does sometimes overlap the nomenclature of other foods with similar characteristics. Fabricated foods are the same as "formulated foods" and "engineered foods". By definition, fabricated foods are made by combining natural or synthetic components, or both, to achieve certain nutritional, physical (textural), chemical, sensory, and stability characteristics. In other words, fabricated foods are ingredients put together in a new form.

Other similar foods may be defined as follows (Bird, 1975):

Simulated foods are designed to completely replace some other food; they are made to look, taste, and feel like the food they replace. Meat analogs, made from plant proteins, are similar to the real meats they replace. Soy milk is designed to replace cows' milk.

Synthetic foods are those made from materials generally thought of as nonfood sources. Artificially sweetened soft drinks and fruit drinks are synthetic fruit juices.

Imitated foods are those made to look like and replace a food that has a "standard", but they do not meet the standard requirements. A sausage-type product that would look, feel, and taste like a frankfurter but does not meet the standard of composition for frankfurters, would be labeled imitation.

Convenience foods requires less labor in storing, handling, preparing, serving or eating than the foods they replace. French fries prepared in a potato-processing plant, canned soups, and sliced bread are examples of convenience foods.

Other nomenclatures such as "textured", "substituted", "converted", "created" have been used in literature for a variety of processes and products with different characteristics. Here we consider only foods prepared from refined macronutrients, rather than those converted from unrefined raw materials.

b. Physico-Chemical Principles

At present we know little about the structures (molecular and supermolecular) of, or interactions between, the basic components which give rise to the various attributes of food products; therefore, it is difficult to design products with predictable compositions, textures, flavors, and storage properties starting from the molecular raw materials (Franks, 1975).

The interaction of biopolymers may, however, falls into several classes: (1) With solvent - hydration - responsible for observed solubility behavior and the stability of "native" states; (2) with non-polymeric species, such as with lipids; (3) with other polymeric species, e.g., protein-carbohydrate, or different types of protein; and (4) with themselves, giving rise to aggregation which may be ordered, as in fibers, or random, as in gels.

As a rule, complex food products are highly heterogeneous, disperse systems and are therefore metastable. They are usually stabilized by "edible surfactants", i.e., proteins, and the nature of the interfacial layers plays an important part in determining the properties of the product.

A very detailed understanding of molecular structure and

conformation can thus help the processer to achieve some degree of control over the interactions of complex polymeric raw materials.

Investigations of the structures and interactions of the various food components in simplified, yet realistic model situations under space conditions may well make it possible to relate molecular details to bulk product attributes.

Tolstoguzov and Braudo (1983) reviewed the aspect of fabricated foods as multicomponent gels. The ability to form gels is characteristic of proteins and polysaccharides. Structuring processes underlying the production of artificial foods may be independent of the type of protein raw material. However, there should be structural compatibility between the filler and the gel matrix. Structural compatibility is the ability of macromolecules or dispersed particles of the protein filler to be distributed in the gel network without distorting it significantly. Tolstoguzov and Braudo (1983) found that, in principle, any process which is intended to give rise to a solid fabricated foodstuff (protein extender or analogue) can be broken down in the following three major stages: (1) the production of a multicomponent liquid system, (2) the shaping of the liquid system, (3) the retention of product shape by converting the liquid system into the gel state.

c. Characteristics

In fabrication the aim is to achieve uniformity, i.e., absence of variability, in the raw materials, and thus a high degree of predictability in product attributes.

Foods will be accepted only when the consumer is satisfied with their taste, odor, texture, and appearance. Among above factors, flavor and texture are the two major characteristics for acceptance. Fabricated foods must be developed to meet the spe-

cific requirements of the ultimate consumer, i.e., space crew, if they are to be successful products.

1) Nutritional Aspects. Nutritional value is a reflection of the physiological needs and wants of the individual consuming it. Thus, the nutritional value of a food product in reality is a very variable, changeable quality depending upon the specific consumer under specific circumstances. Nutritional requirements of the CELSS space inhabitants is discussed under sections III and IV. Since almost all of the foods to be consumed in CELSS will be fabricated from refined macronutrients and selected other additives supplied initially from earth, it is important that the fabricated foods be equal, or superior to, the imitated foods in nutritional value. The value of fabricated foods must be measured on the basis of providing all essential nutrients that contribute to health, not just a relatively few so-called "basic" nutrients.

There is no food which is an absolute necessity in our diet, though some are more important nutritionally than others. Foods are not essential - only nutrients - all 43 of them. As far as our target "vegetarian diet" is concerned, Brigg (1975) believes that one could go "all the way" with soybeans in a totally meatless diet as long as the nutritional differences are otherwise made up and as long as it is justifiable by taste, economics, or the personal desires of the consumer.

Brigg, however, disagrees with using isolated (soy) proteins in fabricated foods and calls it "nutritionally unsound" because of lack of protein deficiency in the American diet, high cost, and its lack of a broad spectrum of vitamins and minerals. Inclusion of such sources, he believes, results in "diluting out" the intake of potassium, folic acid, pantothenic acid, and many other essential nutrients not considered "basic". These cautions are of course not applicable to our exceptional case of food fabrication in the space habitat.

Approaches of measurement of nutritive quality of fabricated foods has been reviewed by Kies (1975):

While fabricated foods must act as carriers of many vitamins, minerals and energy, much of the stress has been on protein quality. Even limiting nutritive evaluation to protein value offers complications. Various interacting factors are involved in determining the value of food products as sources of protein.

Some of the defined direct determinants are as follows:

(1) Amino acid proportionality patterns. Lysine and sulfur-containing amino acid content has received much stress but other interrelationships may well be involved.
(2) Total protein content.
(3) Protein digestibility and amino acid availability.
(4) Total nutritional environment.

Some of the defined indirect determinants are as follows:

(1) Palatability and acceptability. Regardless of the nutritional merits of a product if people won't eat it or if dietary habits limit its inclusion in the diet to very small amounts, its value is nil.
(2) Availability.
(3) Other constituents of the product. These may enhance or detract from value as protein resources.
(4) Other constituents of the food pattern.

Obviously, no single assay procedure could possibly take all these factors into account. An idealized approach to evaluating the value of a supplementary protein would involve a sequential progression from chemical evaluations, to biological evaluations with animals, to biological evaluation with humans, to controlled field and uncontrolled field trials. For routine evaluation of large numbers of test materials such a procedure is prohibitive in terms of time, money and limitations of test materials.

The PER (Protein Efficiency Ratio) method, in spite of its many limitations, is the most popular small animal bioassay method of evaluation of protein quality of food products. When humans are used as the bioassay experimental models, the most popular method of evaluation is the nitrogen balance technique. Kies (1975) has also discussed experiences using humans in the bioassay of Textured Vegetable Protein (TVP) processed to resemble ground beef, a situation very similar to using fabricated foods in space habitat. At 4.0 g N intake/day level, he found a lower protein nutritive value for TVP, which can be overcome at least in part by methionine supplementation. It also indicates methionine as being the first limiting amino acid in the product. At 8.0 g N intake/day level, there was almost identical nitrogen retention, indicating that if sufficient quantities are fed, the TVP can meet the protein nutritional needs of humans. It does suggest, however, that discussion of protein quality in relationship to TVP and beef is really more academic than practical.

When TVP was enriched with 1% D,L methionine, at 4 g N intake/day level results indicated a poorer protein quality for the TVP product in comparison to beef, which could be overcome with methionine supplementation.

Somewhat similar results were found earlier by Bressani et al. (1967). In that study, there wa no evidence of difference in protein quality between skimmilk and a test soybean textured food when fed to children at a protein intake level of 2.0 gm/kg/day. However, an intake of 138 mg N/kg/day from the soy food in comparison to only 97 mg N from skimmilk was necessary for achievement of N equilibrium.

If legal nutritional guidelines regarding the dietary protein quality and quantity minimums are not necessary to follow, then a fabricated food with a PER lower than 2.5 can be made nutritionally equivalent to that of animal products by increasing protein quantity (Table 17).

Table 17
Quantity-Quality Protein Considerations
(from Kies, 1975)

Item	PER	g Protein in 100 g Serving	U.S. RDA Protein in g	Percent U.S. RDA
Animal protein product	2.5	18	45	40
Fabricated analog	2.2	18	65	29
Fabricated analog at higher protein level	2.2	26	65	40

Biological tests for possible presence of toxins, artifacts or loss of amino acids, and protein quality determination are necessary for all fabricated foods before any human feeding study is conducted.

Some type of standard "biological score" will need to be given to their overall nutritional value compared with the product they imitate. This is now done routinely in testing the comparative value of protein used in foods (PER test as an example); but to test for protein quality only, in measuring fabricated foods, is entirely inadequate.

Biological testing of fabricated foods is also essential because of the possible presence of toxic artifacts or loss of amino acids developed in the manufacturing practice - something always possible when proteins are highly processed.

2. Amino Acid Fortification

Rosenfield (1975) believes that the biological value of a fabricated food can be increased by adding the limiting amino acid(s). The FDA on July 26, 1973, finalized the regulation concerning the use of amino acids in foods (FDA 1973). The critical point with respect to amino-acid fortification is that the food must furnish, in a reasonable daily intake, at least 6.5 gm of "naturally occurring primarily intact protein" and the finished food after amino-acid fortification must have a PER of 2.5 or more. On the surface this appears to be a regulatory breakthrough for fabricated protein foods in which the limiting amino acid is methionine. DL-methionine and L-methionine can now be added to foods providing the aforenoted condition is met. In practice, there are taste and odor problems associated with use of methionine which may be not completely solved.

It would seem that the only realistic way at present to raise the PER to 2.5 of fabricated foods which are not highly seasoned and are deficient in methionine is to alter the types and quantities of the protein ingredients. On the other hand, if another essential amino acid such as lysine is the limiting one, then it is possible to fortify the food with the amino acid itself.

The FDA will consider exceptions to the 2.5 PER level only for those cases in which amino-acid addition raises the PER to 80% of that of casein, or effectively 2.0. In some fabricated foods the predominant or sole protein source is wheat gluten. Lysine addition raises the PER of these foods from approximately 1.1 to 1.8. It can be calculated that this increases the utilizable protein by 2 to 3 gm per 100 gm of fabricated food.

3. Vitamin and Mineral Fortification

For CELSS space crew, micronutrients (vitamins and minerals) are initially supplied from the earth. If these nutrients are

not consumed directly as "pills", they could be applied into the fabricated foods, but there appears to be no particular advantage to this approach.

It will be important to assure stability of the vitamin and other supplements by proper packaging and storage conditions as well as in the initial formulation. As an example, the stability of both vitamins B_1 and B_{12} are highly pH dependent and a change in formulation which shifts pH from 6.0 to 6.7-7.0 could increase the rate of destruction of vitamin B_1 by 7 to 10 fold.

Niacin and niacinamide have essentially the same molecular weight and biopotency, and both can be used in most food applications. Both are quite stable. Niacinamide has better solubility in water but lumps readily in handling and storage at ambient relative humidities. The vasodilator effect of niacin does not occur at food use levels, but can affect workers handling the material.

Riboflavin (B_2) and vitamin B_6 are less stable than niacin; with B_2 extremely light sensitive. Suitable market forms of vitamin A palmitate are available for a wide range of applications. These include oil-in-water emulsions, polysorbate-based solution, preisomerized vitamin A palmitate oil, dry water-dispersible beadlets, and powders. A dry, fine particle-size, stabilized form of vitamin A palmitate can be added to conventional flour premixes containing vitamin B_1, vitamin B_2, niacin, and iron and metered continuously into flour streams in the usual way. Vitamin C is sensitive to oxygen, iron, copper, anthocyanin pigments and fortification with vitamin C requires substantial overage in almost all applications to the intended shelf life of the product. Vitamin E, in the form of d-, l-, or d-alpha-tocopheryl acetate, is quite stable in food processing and can be added to foods in a variety of ways Alpha-tocopherol is available commercially, but generally should be used as an antioxidant rather than for fortification.

In all fortification projects on earth, an input or overage above the desired level is essential to ensure compliance after processing and storage. Even in the case of the most stable vitamin, niacin, an overage of approximately 10% is required because of imperfect distribution in its addition to most products and inherent analytical errors in assaying the food. In addition, leaching can cause losses of stable vitamins, e.g., cooking of pasta products, blanching of vegetables. It is usually not difficult to establish a logical overage for each vitamin in each application.

4. Flavoring Fabricated Foods

It is assumed that flavors and spices for fabricated foods (especially fruit flavors and meat analog flavors) are initially supplied from the earth. We have the capability of making totally synthetic fruit flavors with nuances like the freshness of orange juice or the tinniness of canned pineapple (Kuramoto and Katz, 1975). However, development of the optimum meat flavors needs more research.

As good as HVP, autolyzates, MSG, ribotides, and spice blends are in flavoring soups, bouillons, and gravies, they are a long way from the type and quality needed for fabricated meat analogs. For example, in the meat extenders when extension goes beyond 25% to 40 or 50%, meat dilution is so great that enhancers and HVP just do not perform. There is a need also for flavor systems that can be processed as a precursor, so that flavor development occurs when heating ensues.

Hornstein and Crowe (1960) and Wasserman and Spinella (1972) were responsible for delineating which parts of meat are responsible for flavor. Experiments showed that meatiness is derived principally from muscle tissue, and the species charateristic of beef, pork, chicken and the like originate in the fat. More

recent reports by Pepper and Pearson (1971) and Wasserman and Talley (1968) indicate that it is the water-soluble components of adipose tissue that are responsible for species character rather than the fat itself.

Perhaps the most important volatile components resulted from the reaction of H_2S which is produced in part from cystein. Sulfur-containing compounds can then arise from the reactions of H_2S with NH_3, with amino acids, sugars, and fats. Thus, the production of volatile chemicals during heating of muscle tissue appears to be very complex; it involves the reactions of reducing the sugars and sugar phosphates and their Maillard reaction products with amino acids and the Strecker degradation products of amino acids.

A large number of patents are granted. These are essentially flavors by process. It is noteworthy that in many of these patents, thiol groups or thio-forming moieties are important in the eventual formation of meaty flavors (Ohara et al. 1970).

While the previously mentioned chemicals were reported in the analysis of cooked meat, there are many others common to cooked meat that are found also in other heated foods like coffee, nuts, and chocolate. These include aliphatic sulfides, alcohols, enols, and dienals. Many of these contribute organoleptic effects to meat flavor. Other flavor for fabricated foods (e.g., potato, tomato, cheese, chocolate, margarine, coffee lighteners, and whipped toppings) are successfully developed (Kuramoto and Katz, 1975).

As far as the off-flavor of soy protein is concerned, one alternative is to add desirable flavors and try to mask the impact of undesirable flavors (Gremli, 1974; Palkert and Fagerson, 1980). However, flavor can interact with soy protein and thereby decrease the desirable effects. Encapsulation of flavor could possibly solve that problem (Blanchfield and Ovenden, 1974).

5. Other Food Additives

Other than nutritionally important additives and flavors, there are other food additives which are needed in fabricated foods for their specific beneficial results. The major functions of these additives for fabricated foods can be summarized as follows (Glicksman, 1971):

Textures. All foods have shape, texture, form, body, etc. These give the food product its distinct individuality as well as its eating and handling properties. The most important textural additives are probably fats or oils and hydrocolloids (gums and starches). Fats and oils contribute important mouthfeel parameters of richness, creaminess, smoothness, etc. Hydrocolloids control water and sugar components in foods and prevent undesirable crystallization or syneresis. They also control viscosity (thickness) of the comestibles and are essential in allowing foods to be formed or shaped into gels, puddings, sauces, slabs, fibers, etc.

Color. Coloring agents are used in food products primarily to make food appealing and appetizing to the consumer. They are usually inert and nonfunctional although some of the naturally derived colors have been reported to possess antioxidant properties.

Preservation (Stability). One of the earliest uses of additives was for purposes or preservation. It is probable that in the controlled environment of CELSS their use will not be necessary.

6. Role of Carbohydrates

Carbohydrates will be used both for nutritional and for their functional properties in fabricated foods (Glicksman, 1975). The highly soluble common sugars, like sucrose, or in our

case glucose, are used mainly as bodying and sweetening agents. Thus they contribute such bodying attributes as viscosity, texture, density, and mouthfeel, among others, while also imparting sweetness and flavor to the organleptic and sensory aspects of the food.

Carbohydrate polymers, more commonly known as gums and starches (hydrocolloids), are used strictly for their functional properties. These materials are important because of their multi-faceted functional characteristics which can give functionl effects achievable in no other way and at very low concentrations. For example, these materials can tie up 500 parts of water in a rigid gel or product tremendous viscosity changes at concentrations as low as 0.1% (thickening or bodying effects).

The second major property exhibited by a selected few hydrocolloids (e.g., agar, alginate, carrageenan, pectin, starch, etc.) is that of gelling. Gellation is the phenomenon involving the association or cross-linking of the polymer chains to form a 3-dimensional continuous network which traps or immobilizes the water within it to form a firm, rigid structure that is resistant to flow under pressure. Gelation of the above gums varies so widely in gel character and texture that they are used only for specific food applications, and only a few of them can be interchanged in certain applications. In addition to thickening and gelling, gums have many secondary functional properties that are useful in food processing and development.

B. Advantages and Constraints

The use of fabricated foods, especially when the raw material is non-conventional has obvious advantages. In contrast to natural foods it is possible to increase the index of Nutritional Quality or INQ (Assumptions: III-2), and to minimize natural toxic factors. Fabricated foods provide flexibility, since they can be made from a variety of materials and in combinations that are essentially equivalent nutritionally, making it possible to substitute one material for another during periods of shortages of specific raw materials. For instance, the proteins in milk are obtainable only from lactating animals. Fabricated high-protein beverages such as milk analogs (substitutes) can utilize properly processed proteins from soy, wheat, or algae. Another advantage of fabricated foods is that they can be produced with a consistent composition. Furthermore, nutritionally balanced foods can be produced to meet the needs of certain population groups and individuals with specific metabolic problems or specific diseases (such as hypertension, diabetes, or lactose intolerance). Some potential benefits of fabricated foods are summarized in Table 18.

Table 18

Some Potential Benefits of Fabricated Foods

(from Sarrett, 1975)

Provide "complete" diets for special uses (e.g. space foods)
Modify caloric density
Specify ingredients and nutrients
Improve dietary patterns
Avoid disadvantages of some foods
Make better use of protein sources
Utilize by-products
Eliminate naturally occurring toxicants or deleterious compounds
Provide nutritious and time-saving convenience foods
Ensure uniform quality, palatibility, and stability
Provide nutritious snacks that satisfy without excess calories
Supply good nutritive value at lower cost

On the other hand, it is difficult to exactly duplicate a food as it occurs naturally. While this may be of little importance in CELSS the future development of fabricated foods on Earth depends on increasing our understanding of the physical properties of foods.

C. Types of Fabricated Foods

There are two basic types of fabricated foods (Glicksman, 1975): 1) Those designed to simulate natural counterparts (reproducing existing foods) referred to as "analogs", such as meat and dairy analogs, and those which have no counterpart in nature but are prepared to give variety and spice to the diet.

Another division of the fabricated foods is into the following groups (Sarrett, 1975):

- Special dietary foods that constitute the entire diet such as infant feeding formulas and diets for special uses such as control of weight or treatment of metabolic disorders.

- Foods that can replace entire meals such as nutritionally complete beverages.

- Foods that simulate traditional foods (such as margarine, imitation fruit juices, or egg substitute).

- Minor foods such as imitation caviar, whipped toppings, and certain snack foods that ordinarily provide relatively few calories in the diet.

The items of obvious importance in CELSS are those which constitute major portions of the diet. It may be instructive to consider the status of such foods on Earth. In the following section, we will only focus on the meat and dairy analogs as representative of other fabricated foods.

a. Meat Analogs

Various aspects of meat analog fabrication have been reviewed by Smith and Circle (1972), Glicksman (1975), and

Kinsella (1978). Among the many ways to texturize proteins (Table 19), we will only review the important ones.

Table 19
Methods for Texturizing Proteins

Fiber spinning
Extrusion
Chewy gel formation
Autoclaving coagulation
Pressure variation sponge formation
Non-spinning fiber formation
pH treatment of granules

1. Fiber Spinning. Pioneering work on the spinning protein fibers to the preparation of edible meat-like structure was done by Boyer (1954). The process was based on changing the protein configuration by solubilizing the protein, unfolding the protein chains, and reforming them in roughly parallel fashion, thus simulating muscle protein fibers. The process is not widely used.

2. Extrusion. The major technique for fabricating textured vegetable proteins is by extrusion of protein compositions under heat and pressure. This is a simple process compared with spinning and requires much less equipment and technology, consequently seems more appropriate for utilization in CELSS. Extrusion does not produce well-defined fibers but gives fibrous particulates that have good mouthfeel and chewiness similar to meat.

The protein in aqueous dispersion is subjected to heat and

pressure which causes some realignment of the protein molecules as it is extruded from the high-pressure area into the atmosphere, creating a rapid expansion of the product with a rapid flashing off of the water.

A major advantage is that the raw material does not have to be a 90% protein isolate but can be a meal or flour containing much less protein and of particular importance to CELSS the fabrication equipment is simple. The raw material commonly used is solvent-extracted, defatted oilseed meal containing 40 to 70% protein and up to 35% carbohydrate. This material can also be mixed with other texturizing additives prior to extrusion to further modify the processed product. The mixture is subjected to a pressure above 1000 psi and temperatures of 240 to 350°F during processing (Burke 1971).

The equipment is similar to that used for processing thermoplastic resin products, i.e., continuous extrusion cookers. Depending upon the type and condition of the starting material and other factors, the extruded particles may be compacted or expanded. The expanded form is generally preferred.

A typical extrusion apparatus, such as the Wenger Extruder, consists of jacketed chamber housing a screw. At one end of the chamber is a pressure plate with a restricted orifice leading to a narrow tube, at the end of which there is a nozzle. The plastic mass is forced through the extruder by the screw while being heated by steam passing through the jacket and by the pressure in the tube built up by the screw (Rakosky, 1971; Sanderude and Ziemba, 1968).

Mechanical agitation of the screw causes partial oreintation of the protein molecules, and the narrow tube aligns the molecules prior to expansion. While in the tube, the structure of the plastic mass changes and it takes on a fibrious texture. On extrusion through the nozzle, the plastic mass expands to give a continuous, ropy stream of puffed, fibrous, textured protein

which is cut into strips, chunks, or ground into powder or granules.

Protein flour, water, color, flavor, and other functional ingredients are mixed and fed to a cooker extruder. Under pressure and heat, the mixture is extruded and expands. The size and shape of the texturized extrudate is controlled by configuration of the dies and the speed of the cutting knife.

Jet-Cooking Extrusion. One novel extrusion method uses starch carbohydrates as a texturizing base to form various types of novel fabricated products. This process is based on the fact that certain starches of the high-amylose variety have the unique ability to bind other materials into stable extruded shapes. When combined with protein and other nutrients, high-amylose starch-based compositions can be pressure-cooked to give firm textured gels having a chewy, meat-like texture.

The process consists of mixing starch, soy isolate, nutrients, color, and flavoring with water to five a 30% solids dispersion with a protein to starch ratio of about 3 to 1. The mixture is jet-cooked, and, as it comes out of the jet, it can be either cast into blocks or sheets or extruded into cold water, where it sets into fibers or fibrious-like materials. The final gelled material does not disintegrate in either cold or boiling water so that the products can be processed to resemble pieces of meat. The products have textures similar to hamburger or meat loaf and can be used as meat analogs (Anon., 1972; Hullinger et al., 1973).

3. Chewy Gel Formation

A third type of process for creating textured protein foods was developed by Anson and Pader (1957, 1958A, B, 1959). This was a method of making what was called a "chewy gel", essentially a protein-water system of colloidal dimensions. These "chewy

gel" chunks, when chewed, had the physical properties of resilience, elasticity, and resistance to shear. In addition, they had the property of heat stability, i.e., retaining their firmness when subjected to heat, particularly in products which are to be subjected to heat processing or normal cooking conditions prior to eating.

These "chewy gels" are prepared under special conditions of pH, protein concentration, water content, and heat as shown by precipitating a dispersion by pH control and then applying appropriate temperature and pressure to achieve the desired gel. The resultant gel has a smooth, uniform, hydrated structure that is pleasantly moist in the mouth and yet has enough textural firmness to give proper resistance to bite.

Texture and mouthfeel can be modified by incorporating other hydrocolloids into the gel or by coating them on the gel particles. The incorporation of carbohydrate additives makes the gel weaker and less gelatinous. The addition of gums, such as, locust bean gum, reduces the rubberiness of the gel and makes it softer. Alginates, seaweed extracts, and locust bean gum facilitates extrusion and gives products with smoother textural characteristics. Because of their water absorption charactersitics, gums may also be used to modify and control the rehydration properties of a dehydrated gel.

b. Dairy Analogs. Many types of dairy product analogs or substitutes are being fabricated (Glicksman, 1975). Today's many dairy-product simulants have excellent shelf-life and uniform quality. This achievement can be attributed primarily to our greater knowledge of ingredient requirements and application of objective quality control methods for both ingredients and finished products.

Diary products may be employed as guide lines in formulating vegetable-food products. However, the wide scope of ingredients

from which non-dairy products can be made permits imparting properties unique to the product itself. The basic ingredients -- proteins, fats, and carbohydrates -- may be modified to provide them with specific performance properties. Modified vegetable proteins, such as soy, can replace the caseinates. Stabilizers, fats and fillers are modified or selected to give the desired functions in the final product (Arenson, 1969). Functions of dairy analog ingredients are shown in Table 20.

Table 20
Functions of Dairy Analog Ingredients
(Arenson, 1969)

Sugar - imparts sweetness, increases viscosity.
Dextrose - also imparts sweetness, but to a lesser extent than sugar (sucrose).
Corn Syrup - contributes some sweetness and increases viscosity in proportion to syrup D.E.
Corn Syrup Solids - same as for corn syrup.
Malto Dextrin - increases both viscosity and solids content without adding sweetness.
Emulsifiers - promote emulsification for product stability.
Gums - increase viscosity, enhance mouth appeal.
Caseinates - increase viscosity by swelling during pasteurization, assist in structural development.
Vegetable Proteins - same as for caseinates.

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1. Imitation Milk. Attempts to market imitation milks in the U.S. over the past years have failed because of a combination of poor nutritional quality and poor flavor and taste acceptance. However, soybean milk has been prepared and used in the Orient

for hundreds of years. The basic procedure is to soak the bean in water for several hours and then grind them in the water. The mass is then filtered and cooked for 30 minutes to yield a nutritious soy milk beverage (Wilkens and Fang, 1970). In Hong Kong a nutritious soy bean-based soft drink is extremely successful and illustrates the point the synthetic products can be produced that are highly nutritious as well as highly acceptable to the consumer (Hammann, 1969).

A problem with various soy milk products is a characteristic "beany" off-flavor and odor which makes them unpalatable to many consumers. Many processes have been devised to eliminate this flavor problem such as enzymatic treatment, addition of masking chemicals, addition of flavors, etc.

One interesting patent claims a process wherein the soybeans are soaked in water, ground with the addition of an appropriate amount of water, homogenized, pH adjusted to 6.0, mixed with the mould bran of Aspergillus oryzae and related moulds, incubated at 35 to 40°C for a few hours, and then sterilized and homogenized to give a stable odorless soybean milk (Kaisha, 1967). Methods and processes such as this are being continually developed, and eventually good quality soy milk and other vegetable protein milks will become common food commodities.

2. Imitation Cheese. Cheese products have also been recent targets for innovators. Imitation processed cheese and cheese products have been developed experimentally and are typically based on vegetable fats, protein, hydrolyzed cereal solids, buffer salts, color, and flavor. The texture, viscosity, and mouthfeel of soft, processed cheeses and spreads, as well as hard cheeses and grated cheeses, have been reproduced (Horn, 1970).

VII. SUMMARY

The present state of knowledge in food science and technology with respect to capability of producing safe, nutritious and acceptable foods from a limited number of source-independent macronutrients has been reviewed. It seems apparent that production of such fabricated foods for consumption by space crews is feasible, and has several advantages. The advantages include flexibility with respect to source of raw material; capability of maximizing the "index of nutritional quality" with respect to any specific stress conditions, or crew requirements; minimizing the occurrence of naturally occurring toxic, or otherwise undesirable compounds; and consistency of food composition. The disadvantages are primarily due to the necessary development of processing systems capable of performance under space conditions, and the necessity for specification of nutrient requirements of the crew rather than reliance on a more or less incidental fulfillment of nutrient requirement through consumption of an abundant and varied diet, such as that of the majority of U.S. population. However, these disadvantages may be inherent in any CELSS system, not only in one based on engineered foods. Processes for conventional foods are likely to be equally challenging in adaptation to space conditions, and a variety of diets comparable to that feasible on Earth could be achieved in space only through frequent resupply.

For the purposes of the present report the nutritional requirements were based on the Recommended Dietary Allowances of the U.S. Department of Agriculture.

The supply of macronutrients was based on a scenario envisioning the production in the CELSS of two plant species (wheat and soy), and a species of algae. Chemical processes included the conversion of plant waste to sugars, and synthesis of glycerol. Micronutrients and other minor diet components deemed

necessary to well being of the crew were based on resupply or storage of originally supplied materials. The production of an adequate diet from the narrow range of hydroponically, or aeropinically grown plants and algae, and from limited chemical processing seems feasible, and adaptation to major restraints imposed by space conditions is also feasible. (As an example oil extraction from soy would use an aqueous rather than a solvent-based process).

Processes are available for the fabrication of engineered foods from the components assumed generated in the above scenario, but even on Earth these processes have not been optimized sufficiently to produce foods of a quality equivalent to that of the "natural" counterparts. The extent to which simulation or imitation of "natural" foods will be necessary for the presumably highly motivated space crews is not known.

The development of space-compatible food fabrication processes will require a substantial effort both in station simulators, and possibly also in space experiments. This is also true of the development of refining processes for components to be used in the fabricated foods. In as far as fabrication processes are concerned: extrusion, freeze-thaw gel formation, and "chewy gel formation" through precipitation seem suitable for application in space conditions.

Specific instances of engineered food analogs in the U.S. European and Far Eastern industries are known to be successful, and several of these were cited.

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