Kirk-Othmer Encyclopedia of Chemical Technology.

FOODS, NONCONVENTIONAL

Nonconventional foods differ from the usual materials of plant and animal origin used for human food or animal feed (see Feeds and feed additives; Food processing). These materials can be produced from chemical feedstocks, eg, carbohydrates (qv), hydrocarbons (qv), or other industrial organics, by processes such as microbiological, enzymatic, or chemical synthesis, or from existing natural products, containing carbohydrates, proteins (qv), and fats, by physical, chemical, microbiological, or enzymatic modification.

Examples of nonconventional foods include single-cell proteins, ie, dried cells of microorganisms such as algae, bacteria, actinomycetes, yeasts (qv), molds, and higher fungi, or protein concentrates and isolates derived from them; derived plant and animal products, ie, leaf meals and leaf protein concentrates, seed meals and seed meal proteins (see Nuts; Soybeans and other oilseeds), concentrates and isolates of soy, cottonseed, peanut, etc, plant cells grown in tissue culture, fish, and meat protein concentrates and isolates (see Aquaculture chemicals; Meat products); synthetic products, ie, carbohydrates (qv), fats and fatty oils (qv), proteins (qv), peptides, amino acids (qv), and vitamins (qv) prepared by chemical, microbiological, or enzymatic synthesis; and manufactured or combination foods, ie, engineered, restructured, or textured foods, and formulated foods.

Each of these general classes of nonconventional foods has been developed to meet specific applications. For example, single-cell protein (SCP) products provide a source of protein for use in animal feeds in those regions of the world where conventional sources of protein feedstuffs, eg, soybean meal or fish meal, are periodically in short supply or available only at very high prices. SCP products have applications in human foods as protein sources or as functional food ingredients for their flavoring, water- or fat-binding, stabilizing, and thickening characteristics.

Derived plant and animal products make better use or upgrade the nutritional quality of already existing materials or products. Synthetic and manufactured products arose from knowledge of the functional properties of food ingredients and of human and animal nutrition that involved more precise definition of nutrient requirements for growth, reproduction, lactation, and body maintenance in both humans and domestic livestock. Food products have been developed to meet human needs under abnormal environments, eg, military rations for arctic, tropical, or desert environments, and special products for astronauts in space flights.

Numerous reviews have been published on various aspects of nonconventional foods (1–18).

1. Single-Cell Protein

Cells of microorganisms have constituted a portion of human food since ancient times. Yeast-leavened baked products contain the residual nutrients from the yeast cells destroyed during baking (see Bakery processes and leavening agents). Cultured dairy products, such as yogurt, buttermilk, and sour cream, contain up to 10^6 cells of lactic acid bacteria per gram (19) (see Milk and milk products). Other examples of fermented foods consumed since early times include fermented meats, fish, and soybean products.

Modern technology for producing microbial cells for human food or animal feed emerged in Germany during World War I. Baker's yeast, *Saccharomyces cerevisiae*, was grown in aerated tanks using incremental

feeding of molasses as the carbon and energy source, and ammonium salts as the nitrogen source (20) (see Fermentationc). Between World Wars I and II, processes were developed in Germany for producing fats from the sulfite waste liquor of paper (qv) manufacturing using *Endomyces vernalis*, and for producing *Saccharomyces cerevisiae* from wood hydrolysates for use as fodder yeast, ie, the Scholler-Tornesch process. Also during this period, the Heiskenskjold process for propagating *S. cerevisiae* from sulfite waste liquor was introduced in Finland (21, 22).

During World War II, effort was undertaken in Germany to produce food and fodder yeast from waste products such as sulfite liquor. The yeast *Candida utilis*, ie, Torula yeast, grows on pentoses such as D-xylose and D-arabinose present in sulfite waste liquor, as well as on glucose. The Waldhof fermentor, introduced during this period, provided for both agitation and aeration. It was a significant advance in microbial cell production technology, and it enabled improved rates of oxygen transfer to growing cells to occur, resulting in faster growth rates than had been achieved previously.

Two broad classes of microorganisms are of interest (ca 1993) for single-cell protein (SCP) production, ie, photosynthetic organisms, including algae and certain bacteria; and nonphotosynthetic organisms, including bacteria, actinomycetes, yeasts, molds, and higher fungi. In addition, two different uses of SCP are distinguished, ie, food for humans and feed for animals.

1.1. Photosynthetic Organisms

Mass cultivation of algae in ponds or tanks under photosynthetic conditions, using incident sunlight as the energy source and CO_2 as the carbon source, has been investigated in Japan, Taiwan, Mexico, Algeria, India (9, 23–26), and in California in the United States (27, 28). Artificial illumination systems have been used for experimental mass cultivation of algae (29) and in bioregenerative systems for converting CO_2 and human wastes into breathable oxygen and food as part of life-support systems for long-duration space exploration missions (30).

Research has been conducted on growth of blue-green algae heterotrophically in the dark, using organic carbon and energy sources such as glucose or acetate (31–35). The objective of these efforts has been to determine optimum conditions, including pH and substrate concentrations, for high specific growth rates and biomass yields.

Algal cultures must be agitated during growth to maintain cells in suspension and exposure to mutant sunlight, and to remove photosynthesis-inhibiting oxygen. Methods used for agitation include paddle wheels, raceways with arrays of foils to create vortices, and recirculation, either alone or in combination with injection of CO_2 (24, 33) (see Aeration, Biotechnology). Table 1 shows that algal densities in culture ponds are in the range of 1–5 g/L, dry wt basis. Consequently, large volumes of water must be handled in harvesting, dewatering (qv), and drying algal cells.

Centrifugation; flocculation using $Al_2(SO_4)_3$, $Ca(OH)_2$, or cationic polymers; sedimentation; filtration; treatment with ion-exchange resins and drum; sand bed; and sun drying have been investigated for separating, concentrating, and drying algal cells (36, 44–46). All of these methods add significantly to the cost of the product except for sun drying. However, sun drying is difficult to accomplish in humid climates such as in India and southeast Asia.

In California, *Spirulina sp.* grown in paddle-wheel-agitated open ponds with CO_2 is harvested through stainless steel screens, with recycling of the nutrient-rich water to the ponds. The wet *Spirulina* is spray-dried at 60°C for a few seconds to yield a food-grade product (47).

Yields of algae grown in outdoor pond cultures (Table 1) are on the order of $15 - 40 g/(m^2 \cdot d)$ (24 short tons per acre per year). Higher yields can be obtained under artificial illumination, but growth of algae under these conditions is not economically feasible.

Product quality is an important consideration in producing algae for food or feed use. Algal cells must be dried using time–temperature combinations sufficient to destroy pathogenic bacteria and viruses that may

Table 1. Photosynthetic Microorganisms in SCP Production

Organism	Scale	Growth conditions[a]	pH	Yield,[b] g/(m^2·d)	Reference
		Algae			
Chlorella sp.,[f]	plastic ponds[c]	CO_2 or acetate	6.0–7.0	15–40	33
Chlorella ellipsoidea	200-m^2 ponds	outdoor sunlight, continuous CO_2, urea autotrophic or mixotrophic with acetate	6.0–7.0	18.7–27.5	34
Chlorella pyrenoidosa	200-m^2 ponds	outdoor sunlight, continuous CO_2, urea autotrophic or mixotrophic with acetate	6.0–7.0	19.0–30.5	34
	10-L tubular loop bioreactor	CO_2, 2 kPa (0.3 psi) pressure, sunlight, urea fed batch	6.6	26.0–30	35
Scenedesmus acutus	225-m^2 shallow tanks[d]	sunlight, CO_2, sugar cane, molasses (mixotrophic) urea	7.0–8.0	20–25	36
Spirulina maxima	700-m^2 pond	sunlight, 0.5% CO_2	9.0	15	(37, 38)
Spirulina platensis	100-m^2 ponds[e]	$NaHCO_3$, KNO_3	9.5–10.0	22	39
		Bacteria			
Rhodobacter (Rhodopseudomonas) capsulatus	waste ponds[f]	industrial waste substrates, sunlight			(40, 41)
	2-L photobioreactor[g]	artificial light, calcium lactate, 30°C		10.41[h]	42
Rhodocyclus (Rhodopseudomonas) gelatinosus	14-L fermentor[i]	incandescent light, 3.0% wheat bran infusion, 30°C	6.7–7.5	4.33[h]	43

[a]Ambient temperature unless otherwise noted.
[b]Dry wt. To convert $g/(m^2 \cdot d)$ to short tons per $acre \cdot year$, multiply by 1.629.
[c]Cell density of 2.5 g/L.
[d]Strain 276-3A; 20-cm deep.
[e]Fiber glass-stirred ponds, 13–15-cm deep. Cell density of 0.3 g/L.
[f] Cell density of 1.2–2.0 g/L.
[g]Continuous upflow photobioreactor.
[h]g/L.
[i]Continuous fermentor. Cell density of 3.15 g/L.

be present in ponds, particularly in those culture systems based on sewage (see Water, Sewage). The possible presence of heat-stable algal toxins must also be considered. The cyanobacteria (blue-green algae) *Anabaena flos-aquae* and *Microcystis sp* in algal waterblooms on ponds produce toxins which poison farm animals. These toxins are not destroyed by boiling or autoclaving the water (48).

The nitrogen requirements for algal growth can be met by either ammonium salts, urea, or nitrates. Bacterial action in sewage oxidation ponds may also liberate sufficient ammonia for algal growth. Most natural wastes supply sufficient quantities of inorganic nutrients, but additional phosphorus may be needed in some regions for optimal SCP production.

Key factors influencing growth include temperature; pH; availability of CO_2, nitrogen, phosphorus, and other inorganic nutrients; and availability of sunlight as influenced by latitude, cloud cover, and depth of the culture pond or tank. Slow, erratic growth results from wide fluctuations between day and night temperatures in outdoor ponds, and from season to season. Significant amounts of algal biomass may be lost as a result of respiration during the night. In the case of *Spirulina sp.*, this loss may be as great as 35% of the total biomass produced during the day (24).

Table 2. Composition of Photosynthetically Grown Algae, [a]

Organism	Nitrogen	Crude[b] protein	Fat	Ash	Reference
Chlorella sp.	9.3	58	9	3	26
Scenedesmus acutus	8.2–10.2	51.4–63.6	11.2–14.3	7.9–16.7	52
Spirulina sp.	8.8–11.2	55–70	4–7	5–10	47
Spirulina platensis	8.0	50.0	0.5	11.0	53

[a] Dry wt basis.
[b] Crude protein = %nitrogen × 6.25. Does not accurately reflect true protein content. Algal cells may contain nonprotein nitrogen substances, eg, 4–6% nucleic acids, dry wt basis.

The availability of CO_2 and the pH are intimately related because the preferred pH range for growth of many species, such as *Chlorella*, is pH 6.5–7.0 and most of the CO_2 is bound as bicarbonate (HCO_3^-) in solution. Additional CO_2 beyond that present in air (0.03%) must be provided to attain optimum growth.

At Lake Texcoco, Mexico, bicarbonate is available in the alkaline waters from soda ash [497-19-8] (sodium carbonate) deposits (see Alkali and chlorine products). This supply of carbon is adequate for growing *Spirulina maxima*, which tolerates alkaline pH values in the range 9–11 (37, 38). Combustion gases have been used to grow this organism, but this carbon source is not available in many regions (49).

Sunlight availability is critical for algal growth. Outdoor algal cultivation is considered practical only in regions between latitudes 35° N and S, where cloud cover and variations in the length of day and night are minimized (50). Depth of the culture pond also affects availability of sunlight. Research in Israel has shown that as much as 80% of the algal cells in a pond may be in darkness because almost all of the solar irradiation is absorbed in the upper 2–3 cm of liquid depth (39).

Economic evaluations of algal production indicate that production costs vary from $0.15 to $4.00/kg of algal product, depending on type of bioreactor, culture technique, and operating conditions (51). For systems with controlled agitation and carbonation, including raceways and tubular reactors, production costs are estimated to range from $2.00 to $4.00/kg.

Tables 2, 3, and 4 list compositional and nutritional data of selected algae. More extensive compilations on algae are available (26, 58). Algae tend to have lower contents of methionine than is desirable in human and animal nutrition and supplementation with this amino acid is necessary with many species (Table 4).

There is considerable anecdotal information on the history of human consumption of *Spirulina maxima* as a source of protein in Mexico and in the region of Lake Chad in Africa. However, relatively few controlled human feeding studies have been conducted using algae as a significant source of protein in the diet. Consumption of 100 g/d of a mixture of *C. ellipsoidea* and *S. obliquus* resulted in gastrointestinal distress attributed to toxins in the algae (59). Partial substitution of proteins in eggs and fish with *C. pyrenoidosa* did not reduce human nitrogen retention but digestibility was low (60). In other studies, *Scenedesmus* was fed to human subjects at levels up to 20 g/d with no ill effects (61). *Spirulina* was incorporated into diets of hospitalized children in Mexico City and was well tolerated (38). However, consumption of algae in human diets is limited to the extent that nucleic acid intake should not exceed 2 g/d. Higher levels may lead to arthritis and gout.

In general, many species of algae have cell walls resistant to digestive enzymes, dark colors, and bitter flavor. All of these characteristics must be altered to make an acceptable food or feed product.

The principal interest in photosynthetic bacteria for their applicability to SCP production (Table 1) has been in Japan, where *Rhodobacter capsulatus* has been used to treat industrial wastes in sewage ponds (40, 41). The product has been evaluated as a protein supplement in laying hen rations for egg production with acceptable results (40).

Table 3. Amino Acid Composition of Photosynthetically Grown Algae, g/16 g Nitrogen

Amino acid	*Chlorella* sp.[a]	*Scenedesmus* sp.[b]	*Spirulina*sp.[c]	FAO reference pattern[d]
alanine	7.4	7.02	8.28	
arginine	5.74	6.94	7.43	
aspartic acid	8.18	8.34	9.95	
cysteine		1.17	0.93	
glutamic acid	9.74	10.02	13.81	
glycine	5.89	5.13	5.28	
isoleucine	4.00	3.32	6.14	4.2
leucine	8.18	7.11	9.26	4.8
lysine	5.39	5.73	4.93	4.2
methionine	2.26	11.95	2.65	2.2
phenylalanine	4.87	4.14	4.61	2.8
proline	4.35	3.78	4.46	
serine	3.48	3.74	6.30	
threonine	4.18	4.04	5.30	2.8
tryptophan	0.87		1.37	1.4
valine	5.56	4.89	7.00	4.2
nitrogen[e]	9.3	10.2	9.6	

[a]Has 1.91 g histidine/16 g nitrogen. Ref. 26.
[b]Ref. 52.
[c]Ref. 37.
[d]Ref. 54.
[e]Values given are percentages.

1.2. Nonphotosynthetic Organisms

Nonphotosynthetic microorganisms of interest in SCP production include bacteria, actinomycetes, yeasts, molds, and higher fungi. Carbon and energy sources considered for growing these organisms include carbohydrates such as simple sugars, starches, and cellulose (qv); agricultural, forestry, pulp (qv), paper, and food processing wastes containing these carbohydrates; and hydrocarbons and chemicals derived from them, including alcohols and organic acids.

Commercial-scale operations are conducted in batch, fed-batch, or continuous culture systems. Fermentation vessels include the conventional baffled aerated tank, with or without impeller agitation, and the air-lift tower fermentors in which air is sparged into an annular space between the fermentor wall and internal cylinder (1–3). A corrosion-resistant grade of stainless steel (316 L) is usually used for fermentor construction; wood or concrete tanks have been used with agricultural or food wastes.

In batch systems, the concentration of the carbon and energy source for growth is 1–10%. In fed-batch and continuous culture systems, it is usually less than 1% and quantities of nutrients are limited to those required to meet nutritional requirements of the growing organisms. Suitable nitrogen sources include anhydrous ammonia or ammonium salts. Feed-grade phosphate is used as the source of phosphorus. Mineral-nutrient requirements vary among different organisms and are usually added to make up deficiencies in the water supply (see Mineral nutrients). Sulfates are used rather than chlorides to minimize corrosion. Carbon–nitrogen ratios should be 7:1–10:1 for yeasts to favor high protein contents and minimize the fat synthesis in the cells that occurs at higher C:N ratios.

Temperature and pH conditions for optimum growth rates and productivities, ie, dry weight of cells per unit volume per unit time, vary widely but are generally 25–40°C and pH 3.0–7.0, respectively. It is desirable to use strains of microorganisms that tolerate higher temperatures in this range since considerable quantities of

Table 4. Algae Protein Quality and Digestibility[a]

Organism	Treatment[b]	Algae protein, % in diet	Algae protein, % digestibility	PER[c]	NPU[d]	Reference
Chlorella pyrenoidosa (Sorokiniana)		10	86	2.19		55
	0.20% L-methionine	10	86	2.90		55
Chlorella sp.[e]		7.5–15				56
Scenedesmus sp.[g], [f]	0.10% DL-methionine	10				52
Spirulina sp.		10	8.4–8.5	2.2–2.6	53–61	37
Spirulina platensis			75.5		52.7 (68)	57
	0.2% DL-methionine		75.5		62.4 (82.4)	57

[a]Tests on rats unless otherwise noted.
[b]Dried plus addition of indicated compounds.
[c]Protein efficiency ratio (PER) = weight gain(g) for a 10% protein level in the diet of rats as compared to the standard of 2.5 for casein.
[d]Net protein utilization (NPU) = %digestibility(D) × biological value(BV); complete utilization = 100. Biological value is given in parentheses; BV = %of absorbed nitrogen retained in body tissue; complete retention = 100.
[e]Feed/gain ratio of 1.60–1.63.
[f] Tests on chicks.
[g]Feed/gain ratio of 2.0–2.3.

heat are liberated during aerobic growth of microorganisms on either carbohydrates or hydrocarbons. Typical values are 15–34 kJ/g (3.6–8.1 kcal/g) of dry wt cells, depending on yield from a given substrate. In many geographical regions, cooling water is not available at a temperature below 20°C and refrigeration must be provided to control the temperature in the fermentor.

Production of food-grade SCP products requires operation under aseptic conditions in which the air, growth medium, and equipment are sterilized. Feed-grade SCP can be produced under clean but nonsterile conditions provided that a pH of 3.0–4.5 and a large inoculum are used. Transfer of oxygen and substrates to and across the cell surface is an important factor affecting growth rate, yield, and productivity in SCP processes. For yeasts, oxygen requirements range from 1 g/g dry wt of cells with carbohydrates to 2 g/g dry wt of cells for hydrocarbons (62).

Several processes for bacterial SCP production have been developed but abandoned. Imperial Chemical Industries, Ltd. constructed a 50,000–75,000-t/yr plant for producing the bacterium *Methylophilus methylotrophus* from methanol(qv). This process employed an air-lift pressure cycle fermentor, and a proprietary system for separating the cells from the growth medium by agglomeration. This plant is no longer operating because the protein product, Pruteen, was not competitive as an animal feedstuff in west European markets (63).

Large-scale SCP production processes for growing yeasts of the genus *Candida* from hydrocarbon substrates were developed by British Petroleum Co., Ltd. and Kanegafuchi Chemical Industry, Ltd. of Japan (57). However, the 100,000-t/yr capacity plants based on these processes, and constructed in Sardinia and Italy, were abandoned because of regulatory agency questions regarding residual hydrocarbon contents of the products (2, 3).

Table 5 presents typical operating conditions and cell production values for commercial-scale yeast-based SCP processes including (63) *Saccharomyces cerevisae*, ie, primary yeast from molasses; *Candida utilis*, ie, Torula yeast, from papermill wastes, glucose, or sucrose; and *Kluyveromyces marxianus var. fragilis*, ie, fragilis yeast, from cheese whey or cheese whey permeate. All of these products have been cleared for food use in the United States by the Food and Drug Administration (77).

S. cerevisiae is produced by fed-batch processes in which molasses supplemented with sources of nitrogen and phosphorus, such as ammonia, ammonium sulfate, ammonium phosphate, and phosphoric acid, are fed

Table 5. SCP Production Processes Based on Nonphotosynthetic Microorganisms

Organism	Substrate	Scale	Fermentor	Temperature, °C	pH	Cell density,[a] g/L	Specific growth rate,[b] h^{-1}	Yield[c]	Reference
			Bacterial processes						
Methylophilus (Pseudomonas) methylotrophus	methanol	75,000[d]	continuous[e]	35–40	6.0–7.0	30	0.38–0.50	0.50	(64, 65)
			Yeast processes						
Candida (Saccharomycopsis) lipolytica	*n*-alkanes	18,000[f]	continuous	32	5.5	23.6		0.88	(66, 67)
Candida utilis	sulfite waste liquor	30,000[g]		30	4.5		0.5	0.50	5
	ethanol	4,450[d]	plant	30	4.6	6–7	0.3	0.80	68
Hansenula jadinii	sucrose	25,000[g]	continuous	32–35	3.5–4.5	12–150	0.13–0.15	0.52	(69, 70)
Kluyveromyces marxianus var. fragilis	cheese whey (lactose)	56,781[g]	fed-batch	30	4.5		112.5	0.45–0.55	(71, 72)
	cheese whey permeate	1,500[g]	continuous	37	4.6	112.5	0.1–0.3	0.45	73
Saccharomyces cerevisiae	molasses	150,000[g]	fed-batch	30	4.5–5.0	40–45	0.20	0.50–0.54	5
			Mold and fungal processes						
Fusarium graminearum	glucose	1,300[g]	continuous	30	6.0	15–20	0.2	0.53	74
Morchella hortensis	glucose	7,570[g]	batch	25–30	6.5	24–30		0.48	62
Paecilomyces varioti (Pekilo)	spent sulfite liquor	360,000[g]		37	4.5	13	0.14–0.20	0.55	(75, 76)

[a]Dry wt basis.
[b]Dilution rate per h.
[c]g/g of substrate utilized on dry wt basis.
[d]Scale is in t/yr.
[e]Air-lift pressure cycle fermentor.
[f] Scale is in L, working volume.
[g]Scale is in L.

incrementally to meet nutritional requirements of the yeast during growth. Large (150 to 300 m^3) total volume aerated fermentors provided with internal coils for cooling water are employed in these processes (5). Substrates and nutrients are sterilized in a heat exchanger and then fed to a cleaned–sanitized fermentor to minimize contamination problems.

C. utilis yeast is produced by either fed-batch or continuous processes. Aerated–agitated fermentors range up to 300 m^3 total capacity and are operated in the same manner as described for *S. cerevisiae* (2, 5). *C. utilis* is capable of metabolizing both hexose and pentose sugars. Consequently, papermill wastes such as sulfite waste liquor that contain these sugars often are used as substrates.

The Provesteen process, developed by Phillips Petroleum Company, employs a proprietary 25,000-L continuous fermentor for producing *Hansenula jejunii*, the sporulating form of *C. utilis*, from glucose or sucrose at high cell concentrations up to 150 g/L. The fermentor is designed to provide optimum oxygen and heat transfer (69, 70).

K. marxianus var. fragilis, which utilizes lactose, produces a food-grade yeast product from cheese whey or cheese whey permeates collected from ultrafiltration processes at cheese plants. Again, the process is similar

to that used with *C. utilis* (2, 63). The Provesteen process can produce fragilis yeast from cheese whey or cheese whey permeate at cell concentrations in the range of 110–120 g/L, dry wt basis (70, 73).

Molds and higher fungi have been grown in aerated fermentors for food use utilizing a variety of carbohydrates as substrates. Mycelia of various species of mushrooms, such as *Agaricus*, *Lentinus*, *Morchella*, especially *Morchella crassipes*, and *M. hortensis* grow on simple sugars such as glucose or sucrose (62, 63). A process was developed for growing the mycelium of these organisms on a commercial scale in the United States for use as a food-flavoring ingredient rather than as a source of protein. This process is no longer practiced because of the relatively high production costs as compared with the costs of imported dried mushrooms.

Other mold-based SCP processes that have been investigated include utilization of sulfite waste liquor by *Paecilomyces varioti*, conversion of carob bean waste by *Aspergillus niger*, corn- and pea-processing wastes by *Giotrichium sp.*, and coffee-processing wastes by *Trichoderma harzianum* (62). However, none of these processes is practiced commercially.

A product called Myco-protein, based on the continuous aerobic culture of *Fusarium graminearum* with glucose as the substrate, has been developed (74). The nitrogen source fed to the fermentor is gaseous ammonia, which also is used to control pH. Mineral salts required as nutrients are sterilized with the glucose substrate before feeding to the fermentor. The mycelial product is used to form textured protein meat analogues which are sold (ca 1993) on a test-market basis in the United Kingdom.

Dry wt yields of bacteria and yeasts grown on hydrocarbons and methanol are ca 1.0 and 0.5 g/g substrate utilized, respectively. For yeasts, molds, and higher fungi grown on carbohydrate substrates, dry wt yields are 0.5–0.6 g/g substrate utilized. Yeast cells are harvested readily by centrifugation. Molds and higher fungi grow in either pellet or filamentary forms. These organisms can be separated from the growth medium and dewatered by screens, filter processes, or basket centrifuges. It is very costly to separate bacteria from the growth medium by centrifugation because of their small (1–2 μm) size and densities similar to that of water. Bacterial cells can be concentrated by agglomeration or electrocoagulation prior to centrifugation. The resulting wastewater and residual substrates are purified and recycled, particularly in processes based on hydrocarbons, methanol, or ethanol (78).

The product quality considerations for nonphotosynthetic microorganisms are similar to those for algae. Tables 6 and 7 present composition and amino acid analyses, respectively, for selected bacteria, yeasts, molds, and higher fungi produced on a large pilot-plant or commercial scale. Table 8 summarizes results of protein quality and digestibility studies.

Most of the bacteria, yeasts, molds, and higher fungi of interest for SCP production are deficient in methionine and must be supplemented with this amino acid to be suitable for animal feeding or human food applications. Also, lysine–arginine ratios should be adjusted in poultry rations in which yeast SCP is used (62). Human feeding studies have shown that only limited quantities of yeast such as *Candida utilis* can be added to food products without adverse effects on flavor (63).

Nucleic acid contents of SCP products, which range up to 16% in bacteria and 6–11% in yeasts, must be reduced by processing so that intakes are less than 2 g/d to prevent kidney stone formation or gout. Adverse skin and gastrointestinal reactions have also been encountered as a result of human consumption of some SCP products (87).

The FDA regulations provide for the use of dried cells of the yeasts *S. cerevisiae, K. marxianus* var. *fragilis*, and *C. utilis* in foods. Folic acid contents must not exceed 0.04 mg/g (88). Functional concentrates and isolates can be prepared from dried microbial cells by disrupting or removing the cell walls using mechanical means or acid, alkaline, and enzyme hydrolysis; or removing the cell walls and reducing nucleic acid contents by chemical or enzymatic methods. Also, microbial proteins can be spun into fibers (63). Baker's yeast protein concentrate has been approved by the FDA for use as a functional food additive (88).

Table 6. Composition of Nonphotosynthetic Microorganisms Grown on Various Substrates, g/100 g[a]

Organism	Substrate	Nitrogen	Protein[b]	Fat	Crude fiber	Ash	Reference
			Bacteria				
Methylophilus methylotrophus[c]	methanol	13	83	7	<0.05	8.6	64
			Yeasts				
Candida (Saccharomycopsis) lipolytica	*n*-alkanes	10	65	8.1		6	(66, 67)
Candida utilis	sulfite liquor	8.3–8.8	52–55	4.6	2.6	7.3	79
	ethanol	8.3	52	7	5	8	80
Kluyveromyces marxianus var. fragilis	cheese whey	7.2–8.8	45–55	2		6–10	72
	cheese whey permeate	7.6	47	4.6		20	73
Saccharomyces cerevisae	molasses	8.3–8.8	52–55	4.1–5.3		7.1–8.4	5
			Molds and higher fungi				
Fusarium graminearum	glucose	9.6	60	73		6	74
Morchella hortensis	glucose	5.4	34	1.4			62
Paecilomyces varioti[d]	sulfite waste liquor	9.1–10.11	57–63				75

[a]On a dry wt basis.
[b]Protein = % nitrogen $\times$ 6.25.
[c]Energy of approximately 12.6 kJ/g (3.0 kcal/g).
[d]Commonly known as Pekilo.

2. Derived Plant and Animal Products

2.1. Leaf Protein Concentrates

Leaf protein concentrates (LPC) are prepared by crushing plant material, extracting the juices, and either using the juice per se or recovering the protein from the juice by heating or chemical precipitation. Dehydrated alfalfa (lucerne) has a long history of use as a source of plant protein for animal feeds. The leaves of alfalfa and other crops are a source of protein that can be extracted to give a concentrated product having increased protein and decreased fiber contents suitable for animal feeding. Plants, other than alfalfa, considered as sources of LPC include pea vines, clover, field beans, mustard, kale, fodder radish, banana leaves, and aquatic plants (89, 90). LPC production requires crops having rapid growth and high yields or protein during the growing season, ie, 1600 kg/hm^2 (1430 lbs/acre); absence of mucilaginous sap which makes it difficult to separate juice from the fiber; absence of acidic or high tannin saps which prevent extraction of protein into the juice because of precipitation in the pulp; and absence of toxic materials such as cyanogenic glycosides, glucosinolates, and alkaloids that can be carried into the final product (91). In addition, from an economic standpoint, the entire plant must be utilizable for LPC.

2.2. LPC Processes

Process development for LPC production dates from the United Kingdom and Hungary from 1920–1940 (89, 90). Table 9 presents some of the processing methods that are used or under development in the 1990s.

Various mechanical methods can be employed for rupturing leaf cells to prepare LPC (95). Leaf structural factors affecting protein release by mechanical processes include leaf weight, cell numbers, leaf thickness, intercellular space, and protein content as a function of leaf maturity. Dynamic compression is considered to be superior to shearing for commercial scale leaf rupturing processes. Other studies have shown that screw expellers should be modified to provide angled paddles for disintegrating leaves before they are passed into

Table 7. Amino Acid Content of Nonphotosynthetic Microorganisms Grown on Various Substrates, g/16 g N[a]

Organism	Substrate	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Try	Tyr	Val	Reference
								Bacteria												
*Lactobacillus bulgaricus*2217		9.0	4.5	10.5	0.4	9.1	3.5	2.2	4.5	6.1	9.3	2.2	3.2	3.6	2.6	4.3		3.3	5.8	81
Lactobacillusdelbrueckii B443		7.5	4.6	14.6	0.5	12.4	4.3	2.0	5.5	7.6	9.6	1.9	4.2	3.3	2.8	4.4		3.3	5.9	81
Methylophilus (Pseudomonas) methylotrophus[b]	methanol	6.8	4.5	8.5	0.6	9.6	4.9	1.8	4.3	6.8	5.9	2.4	3.4	3.0	3.4	4.6	0.9	3.1	5.2	64
								Yeasts												
Candida (Saccharomycopsis) lipolytica[c]	*n*-alkanes	7.4	4.8	10.2	1.1	11.3	4.8	2.0	4.5	7.0	7.0	1.8	4.4	4.4	4.8	4.9	1.4	3.5	5.4	66
Candida utilis[d]	sulfite waste	5.8	5.4	9.2		15.6	3.6	1.2	3.8	7.6	4.8	1.1	8.6	6.0	5.0	5.4	2.4	6.2	3.8	5
	ethanol	5.5	5.4	8.8	0.4	14.6	4.5	2.1	4.5	7.1	6.6	1.4	4.1	3.4	4.7	5.5	1.2	3.3	5.7	80
Kluyveromyces marxianus var. fragilis	cheese whey							2.1	4.0	6.1	6.9	1.9	2.8			5.8	1.4	2.4	5.4	80
	cheese whey permeate	7.1	4.3	7.4	0.3	14.9	3.7	1.8	3.7	5.8	6.0	1.0	3.2	3.0	4.0	4.7	2.6	0.9	4.5	72
Saccharomyces cerevisiae	molasses		5.0		1.6			4.0	5.5	7.9	8.2	2.5	4.5			4.8	1.2	5.0	5.5	5
								Molds												
Fusarium graminearum[e]		6.3	5.4	8.4	0.5	11.9	4.3	2.1	3.5	4.6	6.1	1.6	4.5	4.5	4.5	4.3	1.8	3.1	4.9	74
Morchella hortensis	glucose	4.5	4.0	4.6	0.4	15.4	3.0	1.9	2.4	5.0	3.0	0.7	2.3	4.5	2.8	2.7	1.0	1.9	2.9	62
Paecilomyces varioti	spent sulfite liquor				1.1				4.3	6.9	6.4	1.5	3.7			4.6	1.2	3.4	5.1	75
FAO reference									4.2	4.8	4.2	2.2	2.8				1.4		4.2	82

[a]Dry wt basis.
[b]Commonly called Pruteen.
[c]Commonly called Toprina.
[d]Commonly called Torula.
[e]Commonly called Myco-protein.

the pressing section (89). Heating and drying conditions used during the processing of LPC must be controlled carefully to minimize nonenzymatic browning, Maillard reactions, and reactions between the proteins and unsaturated fatty acids.

Solvent extraction removes chlorophyll and other pigments to give a light-colored product but increases processing costs. Furthermore, solvent extraction removes β-carotene and reduces vitamin A activity (89) (see Terpenoids; Vitamins). Supercritical CO_2 extraction at 30 and 70 MPa (4,350 and 10,150 psi) and 40°C removed 90 and 70% carotene and lutein, respectively, from alfalfa LPC (96). This process avoids organic solvent residues and recovers valuable by-products.

Leaf materials also contain lipoxidases and highly unsaturated lipids. LPC process conditions should inactivate lipoxidases to obtain a stable product (92, 97).

The USDA Western Regional Research Center has developed an improvement to the Pro-Xan process (Table 9) for preparing a bland, colorless LPC product from alfalfa suitable for human consumption (92, 93, 98). Aqueous sodium metabisulfite [7681-57-4], $Na_2S_2O_3$, is added to alfalfa prior to expressing the juice to lighten the color of the LPC and protect cystine and methionine from oxidation. The juice, after expressing, is heated by steam injection to 60–65°C for 10–20 s and cooled to 45°C. The proteins associated with the chloroplasts are

Table 8. Protein Quality and Digestibility of Nonphotosynthetic Microorganisms

Organism[a]	Substrate	Animal	Microorganism in diet, %	Protein digestibility, %	PER[b]	BV,[c] %	Feed conversion ratio,[d] kg/kg wt gain	Reference
Bacteria								
Methylophilus methylotrophus	methanol	chicken	9.8				2.3 (2.33)	64
		pig	6.7				3.13 (3.34)	
Yeasts								
Candida lipolytica	*n*-alkanes	rat		96		61		78
		rat[e]		96		91		78
		chicken	10	88			2.58 (2.68)	78
		pig	7.5	92			3.04 (3.11)	78
		rat		85–88	0.9–1.4	32–48		83
Candida utilis	sulfite waste liquor	rat[f]		90	2.0–2.3	88		83
	ethanol	rat	8		2.10			84
Kluyveromyces marxianus var. fragilis	cheese whey	rat			2.26			71
Saccharomyces cerevisiae	molasses	rat			2.63			84
Molds								
Fusarium graminearum		rat			2.4			85
		rat[g]			3.4			85
		human				8.4		85
		pig	40				1.76	86

[a]Dried plus addition of indicated compound.
[b]Protein efficiency ratio (PER). See Table 4.
[c]Biological value(BV) = % of absorbed nitrogen retained in body tissue; complete retention = 100.
[d]Data in parentheses for control group, with no single-cell protein in diet.
[e]0.3% DL-methionine added to feed.
[f] 0.5% DL-methionine added to feed.
[g]Methionine added to feed.

Table 9. Selected Processes for Leaf Protein Concentrate Production, 1993

Process	Description	Reference
Rothamsted	pulping in ribbed rollers; precipitating protein at80°C or at pH 4.0; drying in air below 80°C	90
Pro-Xan	chopping; ammoniation to pH 8.5; roll or twin-screw pressing; sieve purification; coagulation with steam at 85°C; dewatering; drying	(92, 93)
Vepex	mechanical disintegration; multistage pressing;add-back of liquor to press cake; coagulation at82°C with addition of flocculents; centrifugation;evaporation; drying	94
Instituto di Industrie Agarie[a]	chopping and screw pressing; centrifugation;coagulation at pH 8.5; treatment withpolyelectrolyte; centrifugation; precipitation atpH 4.0; centrifugation; drying	94

[a]Pisa, Italy.

Table 10. Analysis of Leaf Protein Products, wt [a]

Leaf protein	Source	Protein[b]	Fat	Crude fiber	Ash	Reference
white LPC[c]	alfalfa	88.7	0.6	1.0	0.4	93
white LPC	quinoa	93.2	0.7	0.9	2.0	99
Pro-Xan[d]	alfalfa	61.9	8.9	1.7	11.1	93
Brassica napus[e]		58.5	15.6	2.0	10.0	100

[a] Dry wt basis.
[b] $\text{Protein} = \%\ \text{nitrogen} \times 6.25$.
[c] $\text{Nitrogen} - \text{free extract} = 9.3\%$, and $\text{soluble solids} = 0.3\%$.
[d] Whole leaf protein concentrate, 16.5% nitrogen-free extract and 7.9% soluble solids.
[e] Commonly known as Late Korean rape.

then removed by centrifugation. The suspended solids are removed from the liquid phase in the centrifuge by a plate-and-frame filter press, and the proteins in the filtrate are precipitated at 80°C for 2–4 min. The protein precipitate is removed by centrifugation, and the protein is washed at pH 4–5 and spray dried to give a white LPC having potential food applications. The chloroplast protein fraction is adjusted to pH 8.5, and heated to 90–95°C to coagulate the protein. The protein is dewatered by centrifugation, granulated, and dried in a rotary dryer to give the feed-grade Pro-Xan II LPC (93, 98).

The Vepex process developed in Hungary (Table 9) involves disintegration of plant materials followed by double screw pressing to maximize juice production. Green chloroplastic protein is removed by direct steam-injection heat treatment at 82°C with the addition of flocculents and centrifugation. The white protein fraction is separated from the chlorophyll-free process juice by direct steam injection at 80°C, followed by centrifugation and drying (94).

2.3. LPC Product Quality

Table 10 gives approximate analyses of several LPC products. Amino acid analyses of LPC products have been published including those from alfalfa, wheat leaf, barley, and lupin (101); soybean, sugar beet, and tobacco (102); Pro-Xan LPC products (100, 103); and for a variety of other crop plants (104, 105). The composition of LPCs varies widely depending on the raw materials and processes used. Amino acid profiles are generally satisfactory except for low sulfur amino acid contents, ie, cystine and methionine.

Enzyme degradation of leaf protein may occur during crushing and separation from the fiber. The amino acids produced by this enzyme action are soluble in the juice and may be lost unless all of the juice is recovered.

Table 11 presents data on the protein quality of a variety of LPC products obtained from rat-feeding studies. Typical protein efficiency ratio (PER) values for LPCs derived from alfalfa range from 1.41 without supplementation to 2.57 with 0.4% methionine added; casein can be adjusted to a PER of 2.50 (98, 100). Biological values (BV) of mixtures of LPCs, such as barley and rye grass or soybean and alfalfa, may be higher than either LPC alone. The effect has been attributed to the enhanced biological availability of lysine in these mixtures (99).

Human-feeding studies on LPC have been conducted in Jamaica. Diets for malnourished infants contained half of the nitrogen as LPC, and nitrogen retention was equivalent to that obtained with milk (107). An LPC derived from alfalfa has been shown to alleviate the symptoms of the protein deficiency disease Kwashiorkor (108). In India, LPC added to low protein diets, ie, 12.6 g/d protein, to provide an intake of 45–48.8 g/d protein resulted in improved nitrogen retention and digestibility in 10–12 yr-old children (107). Allergic reactions including facial edema were common in 1 out of 8 children. There are definite limits to the use of LPC products in human diets, and raw materials used for LPC production must be evaluated for possible allergenic problems. There is a lack of published information on the evaluation of the nutritional value of white LPCs in human diets.

Table 11. Nutritive Value of Leaf Protein Concentrates and Other Protein Products[a]

Product	True digestibility, %	PER[b]	BV[c]	NPU[d]	Reference
	Leaf protein				
alfalfa Pro-Xan	77.9	1.41			100
alfalfa white LPC	91		59	54	99
Brassica carinata[e]	84	2.12			100
Brassica napus	72	1.80			100
barley	88		63	55	106
barley plus ryegrass LPC	81		73	59	106
soybean	90		55	49	99
soybean plus alfalfa white LPC	90		62	56	99
	Other protein				
fish meal	88		54	48	99
skim milk	96		52	50	99

[a]Rat-feeding studies. LPC = leaf protein concentrate.
[b]Protein efficiency ratio (PER) = weight gain (g) for a 10% protein level in the diet of rats as compared to the standard of 2.5 for casein.
[c]Biological value(BV) = % of absorbed nitrogen retained in body tissue; complete retention = 100.
[d]Net protein utilization(NPU) = % digestibility × BV; complete utilization = 100.
[e]Commonly known as Ethiopian mustard.

2.4. Functional Properties of LPCs

LPC products prepared from *Brassica sp.*, soybeans, sugar beets, and tobacco have been investigated for their functional properties including nitrogen solubility, fat- and water-binding capacity, emulsification, gelation, and foaming capacity and stability (102, 109). The emulsification properties and foam stability of alfalfa LPC indicate potential applications in salad dressings and as a substitute for egg whites in baking (109).

Vegetable oil gels can be formed from a heated emulsion of alfalfa LPC and peanut oil (110). However, the flavor and texture of these gels are not generally acceptable. In general, whole green LPCs suffer from undesirable sensory properties. White LPCs, from which chlorophyll, phenolic compounds, and flavonoid pigments are removed, have desirable sensory properties and can be prepared by the process described previously. However, no economically viable processes have been developed for food-grade white LPC production.

The 1993 market for LPC-type products in the United States was for dried alfalfa meal for animal feed. This product is sold for both protein and carotenoid content. The USDA Pro-Xan product attempts to obtain improved xanthophyll contents for use in egg-laying rations in addition to protein contents. The limitations to commercial development of LPC products for human food use are high capital costs as compared with the low yields of protein, seasonal availability of raw materials, and the need in the United States for FDA approval of the products.

2.5. Seed-Meal Concentrates and Isolates

Seed-meal protein products include flours, concentrates, and isolates, particularly soy protein products. These can be used as extenders for meat, seafood, poultry, eggs, or cheese (see Soybeans and other oilseeds). Detailed information on soybean and other seed-meal production processes is available (13, 14, 18).

Soybean concentrate production involves the removal of soluble carbohydrates, peptides, phytates, ash, and substances contributing undesirable flavors from defatted flakes after solvent extraction of the oil. Typical concentrate production processes include moist heat treatment to insolubilize proteins, followed by aqueous extraction of soluble constituents; aqueous alcohol extraction; and dilute aqueous acid extraction at pH 4.5.

Commercial soy protein concentrates typically contain 70 to 72% crude protein, ie, nitrogen $\times$ 6.25, dry wt basis. Soy protein isolates are prepared from desolventized, defatted flakes. A three-stage aqueous countercurrent extraction at pH 8.5 is used to disperse proteins and dissolve water-soluble constituents. Centrifugation then removes the extracted flakes, and the protein is precipitated from the aqueous phase by acidifying with HCl at pH 4.5. The protein precipitate is washed with water, redispersed at pH 7, and then spray dried. Typical commercial soy protein isolates contain greater than 90% crude protein, dry wt basis.

A modification of the conventional soy protein isolate process has been investigated on a small pilot-plant scale. It is based on the absorption of water from the aqueous protein after extraction at pH 8.5 using temperature-sensitive polyisopyropylacrylamide gels, followed by spray drying to give a 96% protein isolate (111).

Soy protein concentrates and isolates can be formed into fibrous structures by twin-screw extrusion texturization processes. The functional characteristics of these structures are influenced by pH adjustment during processing (112). Soybean protein also can be formed into fibers by forming a spinning dope from a slurry at pH 10–11, which is then aged at 40–50°C. This slurry is forced through a spinneret into an acid-coagulating bath. The fibers are heated to reduce the diameter to about 75 μm. The fibers can be formed with binders (eg, egg albumin), colored, and flavored to give the desired product characteristics (113).

Products prepared from soy protein products and resembling chicken, ham, frankfurters, and bacon are available commercially. Soy protein isolates are used in place of milk proteins or sodium caseinate in products such as coffee creamers, whipped toppings, yogurt, and infant formulas (see Dairy substitutes). Soy protein products also are used in snacks and in baked foods.

Hydration; water, fat, and flavor binding; gelation; emulsifying; foaming; and whipping characteristics vary among different soy protein products and complete substitution of animal proteins by these products is not always possible (114).

Soy protein products may impart a beany flavor to foods when used at levels greater than 20%. Undesirable components are present in the beans prior to processing and also may be generated during processing. Off-flavors and odors also may arise from oxidation of lipid components and from degradation of phenolic compounds during thermal processing of foods containing soy proteins. These undesirable flavor components can be diminished, but not completely eliminated, by extraction with alcohols such as methanol, ethanol, or isopropanol, or with an azeotropic mixture of hexane and an alcohol (115).

Vegetable proteins other than that from soy have potential applicability in food products. Functional characteristics of vegetable protein products are important factors in determining their uses in food products. Concentrates or isolates of proteins from cotton (qv) seed (116), peanuts (117), rape seed (canola) (118, 119), sunflower (120), safflower (121), oats (122), lupin (123), okra (124), and corn germ (125, 126) have been evaluated for functional characteristics, and for utility in protein components of baked products (127), meat products (128), and milk-type beverages (129) (see Dairy substitutes).

Functional properties of canola protein products can be improved by succinylation (130, 131). Controlled acetylation can reduce undesirable phenolic constituents as well (132). However, antinutrients in canola and other vegetable protein products such as glucosinolates, phytic acid, and phenolic compounds have severely limited food applications of these products.

2.6. Hydrolyzed Vegetable Protein

To modify functional properties, vegetable proteins such as those derived from soybean and other oil seeds can be hydrolyzed by acids or enzymes to yield hydrolyzed vegetable proteins (HVP). Hydrolysis of peptide bonds by acids or proteolytic enzymes yields lower molecular weight products useful as food flavorings. However, the protein functionalities of these hydrolysates may be reduced over those of untreated protein.

Deamidation of soy and other seed meal proteins by hydrolysis of the amide bond, and minimization of the hydrolysis of peptide bonds, improves functional properties of these products. For example, treatment of

soy protein with dilute (0.05 *N*) HCl, with or without a cation-exchange resin (Dowex 50) as a catalyst (133), with anions such as bicarbonate, phosphate, or chloride at pH 8.0 (134), or with peptide glutaminase at pH 7.0 (135), improved solubility, whipability, water binding, and emulsifying properties.

HVP products prepared by hydrolysis with HCl contain varying amounts of glycerol chlorohydrins, such as 3-chloro-1,2-propanediol [96-24-2] and 1,3-dichloro-2-propanol [96-23-1], depending on reaction conditions and lipid contents of the starting material (135). As a result of their toxicities, regulating agencies in many countries have restricted the contents of these compounds in food.

Under FDA regulations, HVP products are permitted as optional ingredients in standardized canned foods such as pears, mushrooms, and tuna, and as a flavoring ingredient in nonstandardized foods (137). The U.S. Department of Agriculture has cleared HVP as a flavoring ingredient in various meat products (138).

2.7. Fish Protein Concentrates and Isolates

Fish protein concentrates (FPC) and isolates (FPI) are produced for human food use from whole edible species of fish using sanitary processing methods; fish meal and fish solubles are produced for animal feed. FPC raw materials include whole hake, hake-like fish, and herring of the genera *Clupea*, and menhaden and anchovy of the species *Engraulis mordax* without removal of heads, fins, tails, or intestinal contents. FPI raw materials include edible portions of fish body generally recognized as safe for human consumption after removal of heads, fins, tails, bones, scales, viscera, and intestinal contents (139). In the United States, FDA regulations describe the production processes for preparing FPC and FPI (139). The FDA regulations also specify that FPC and FPI contain minimum protein contents of 75 and 90%, respectively, a maximum fat content of 0.5%, and a maximum moisture content of 10% by weight. FPC must be free of *Escherichia coli*, *Salmonella*, and other food pathogens and have a total bacterial plate count of not more than 10,000 per gram.

Amino acid profiles of FPC are excellent and compare favorably with whole egg except for tryptophan and lysine (140). Hake and Atlantic FPCs prepared by isopropanol extraction have PERs of 3.29 and 3.05, respectively, as compared with 3.0 for casein (140). Numerous human feeding studies have been conducted with FPC. The results indicate that high quality, bland FPC products can be used as protein supplements but they are not suitable for use as a sole source of protein.

Fish protein concentrates vary widely in functional characteristics, ranging from those having high protein content and low water solubility, to those having lower protein contents but improved water solubility. Attempts have been made to improve functional properties of fish protein by enzyme hydrolysis (141), or by modification of the myofibrillar protein by succinylation (142).

Economic conditions in the United States have not favored the production of FPC and FPI having desirable functional and nutritional characteristics at prices competitive with those of conventional protein sources.

2.8. Textured and Structured Fishery Products

Numerous seafood analogue products, eg, crab, shrimp, and lobster analogues, have been prepared by modifying the structural and textural properties of fish proteins. Surimi, originally developed in Japan, is prepared from mechanically deboned fish muscle, such as Alaska pollock (*Theragara chalcogramma*), by freshwater leaching to yield a light-colored, bland, refined protein that can be used as a matrix for seafood analogues.

A typical process for manufacturing surimi-based seafood analogue products involves (143) mincing Alaska pollock; washing it at 10°C with water having pH 6.5–7.0 and low Ca^{2+}, Mg^{2+}, Fe^{2+}, and Mn^{2+} contents; rinsing, draining, and screw-press dewatering to 82% moisture; adding sucrose and sorbitol (91:1) to a final concentration of 9% as cryoprotectants; fabricating with starch, egg white, lactalbumin, and fat or oil to give the desired texture; incorporating flavoring ingredients; and cooking.

In addition to sucrose and sorbitol, polydextrose can be used as a cryoprotectant (144) (see Sugar alcohols). Also, the type of starch used, ungelatinized or pregelatinized, affects the extent of water binding by surimi gels

during mixing and cooking (145). More detailed information on the technologies for manufacturing seafood analogues is available (15, 16).

A number of investigations have been directed toward improving the functional characteristics of fish proteins by enzymatic hydrolysis. Treating comminuted and defatted sardines (*Sardina pilchardus*) with subtilisin (Alcalase), to give a 5% degree of hydrolysis, solubilized the proteins and gave a product having improved emulsifying properties over those of sodium caseinate (146).

Eviscerated and ground mullet (*Mugil cephalus*) was hydrolyzed with bacterial alkaline proteases without adding water, followed by centrifugation to remove 80% of the liquid, and drying (147). A high degree of protein solubilization (70–80%) was achieved in the final product, which contained 83 to 86% protein. However, rat-feeding studies indicated that the hydrolysates had about 10 to 15% lower nutritional value than that measured by PER and feed efficiency values, ie, feed consumed/weight gain.

3. Synthetic Protein Products

3.1. Plastein Synthesis

Plasteins are mixtures of high molecular weight proteinaceous peptides. They are synthesized by enzyme-catalyzed growth of peptide chains from lower molecular weight peptides. The process by which plasteins are formed is called the plastein reaction and is the reverse of the proteolytic enzyme hydrolysis of peptide bonds of proteins (148). Japanese investigators have conducted extensive studies on the utility of the plastein reaction and of plasteins in food technology. The enzymatic modification of proteins from such products as soybeans, codfish, algae (*Chlorella*), wheat, milk, and baker's and hydrocarbon-grown yeasts followed by plastein synthesis have been investigated (149, 150). The plasteins prepared were bland and did not have objectionable tastes, odors, or colors.

Plasteins are formed from soy protein hydrolysates with a variety of microbial proteases (149). Preferred conditions for hydrolysis and synthesis are obtained with an enzyme-to-substrate ratio of 1:100, and a temperature of 37°C for 24–72 h. A substrate concentration of 30 wt %, 80% hydrolyzed, gives an 80% net yield of plastein from the synthesis reaction. However, these results are based on a 1% protein solution used in the hydrolysis step; this would be too low for an economical process (see Microbial transformations).

Fish protein concentrate and soy protein concentrate have been used to prepare a low phenylalanine, high tyrosine peptide for use with phenylketonuria patients (150). The process includes pepsin hydrolysis at pH 1.5; pronase hydrolysis at pH 6.5 to liberate aromatic amino acids; gel filtration on Sephadex G-15 to remove aromatic amino acids; incubation with papain and ethyl esters of L-tyrosine and L-tryptophan, ie, plastein synthesis; and ultrafiltration (qv). The plastein has a bland taste and odor and does not contain free amino acids. Yields of 69.3 and 60.9% from FPC and soy protein concentrate, respectively, have been attained.

A pepsin hydrolysate of flounder fish protein isolate has been used as the substrate (40% w/v) for plastein synthesis, using either pepsin at pH 5 or alpha chymotrypsin at pH 7, with an enzyme–substrate ratio of 1:100 w/v at 37°C for 24 h (151). The plastein yields for pepsin and alpha chymotrypsin after precipitation with ethanol were 46 and 40.5%, respectively.

Fish silage prepared by autolysis of rainbow trout viscera waste was investigated as a substrate for the plastein reaction using pepsin (pH 5.0), papain (pH 6–7), and chymotrypsin (pH 8.0) at 37°C for 24 h (152). Precipitation with ethanol was the preferred recovery method. Concentration of the protein hydrolysate by open-pan evaporation at 60°C gave equivalent yields and color of the final plastein to those of the freeze-dried hydrolysate.

The sulfur amino acid content of soy protein can be enhanced by preparing plasteins from soy protein hydrolysate and sources of methionine or cystine, such as ovalbumin hydrolysate (plastein AB), wool keratin hydrolysate (plastein AC), or L-methionine ethyl ester [3082-77-7] (alkali saponified plastein) (153). Typical

PER values for a 1:2 mixture of plastein AC and soybean, and a 1:3 mixture of alkali-saponified plastein and soybean protein, were 2.86 and 3.38, respectively, as compared with 1.28 for the soy protein hydrolysate and 2.40 for casein.

Plasteins are still in the experimental stage of development. Further work is needed on the scale-up of processing conditions for plastein synthesis which would lead to commercially useful products and on the functional utility of plasteins as ingredients in foods.

3.2. Synthetic Proteins

Protein-like polypeptides can be synthesized chemically from ammonia, water, and carbon dioxide, or from mixtures of amino acids which are now manufactured by chemical or microbiological synthesis (154). Polyamino acids can be produced in the laboratory by simultaneous polymerization of mixtures of amino acids at 180°C for 3–6 h under dry conditions. Protenoids, containing all of the common amino acids in peptide linkages, can be obtained if sufficient amounts of aspartic and glutamic acids are included in the reaction mixture. Apparently these protenoids are digestible by mammalian proteinases and can serve as sources of nutrients for the bacteria *Lactobacillus arabinosus* and *Proteus vulgaris*. The possibility exists for protenoids to be nutritionally imbalanced, have mammalian toxicity, and undesirable tastes, odors, and stability. These problems must be investigated further before any assessment of the utility of polyamino acids or protenoids can be made. No progress has been made in the early 1990s on developing useful food protein ingredients by purely chemical synthetic methods.

4. Product Quality and Safety

The Protein Advisory Group, ad hoc, is the working group of the WHO United Nations system involving WHO, FAO, and the United Nations International Children's Emergency Fund (UNICEF). It has developed guidelines for the evaluation of novel sources of protein, eg, single-cell protein; clinical testing of novel sources of protein; human testing of supplementary food mixtures; and nutritional and safety aspects of novel protein sources for animal feed (155).

In general, nonconventional protein foods must be competitive with conventional plant and animal protein sources on the bases of cost delivered to the consumer, nutritional value to humans or animals, functional value in foods, sensory quality, and social and cultural acceptability. Also, requirements of regulatory agencies in different countries for freedom from toxins or toxic residues in single-cell protein products, toxic glycosides in leaf protein products, pathogenic microorganisms, heavy metals and toxins in fish protein concentrates, or inhibitory or toxic peptide components in synthetic peptides must be met before new nonconventional food or feed protein products can be marketed.

In the United States, novel food ingredients or food ingredients produced by novel processes must be cleared by the FDA. In the case of meat and poultry, novel ingredients must also be cleared by the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS).

BIBLIOGRAPHY

"Proteins from Petroleum" in *ECT* 2nd ed., Suppl. Vol., pp. 836–854, by E. R. Elzinga and A. I. Laskin, Esso Research and Engineering Co.; "Foods, Nonconventional," in *ECT* 3rd ed., Vol. 11, pp. 184–207, by J. H. Litchfield, Battelle, Columbus Laboratories.

Cited Publications

1. I. Goldberg, *Single Cell Protein*, Springer-Verlag, Berlin, 1985.
2. J. H. Litchfield in J. L. Marx, ed., *A Revolution in Biotechnology*, Cambridge University Press, Cambridge, U.K., 1989, 71–81.
3. D. Sharp, *Bioprotein Manufacture: A Critical Assessment*, Ellis Horwood, Chichester, U.K., 1989, p. 140.
4. I. Goldberg and R. Williams, eds., *Biotechnology and Food Ingredients*, Van Nostrand Reinhold Co., Inc., New York, 1991.
5. G. Reed and T. W. Nagodawithana, *Yeast Technology*, 2nd ed., Van Nostrand Reinhold Co., Inc., New York, 1991.
6. A. Halasz and R. Lasztity, *Use of Yeast Biomass in Food Production*, CRC Press, Boca Raton, Fla., 1990.
7. M. Moo-Young and K. F. Gregory, eds., *Microbial Biomass Proteins*, Elsevier Applied Science Publishers, Ltd., London, 1986.
8. A. Richmond, *CRC Handbook of Microalgae Mass Culture*, CRC Press, Boca Raton, Fla., 1986.
9. G. Shelef and C. J. Soeder, eds., *Algae Biomass Production and Use*, Elsevier, Amsterdam, the Netherlands, 1980.
10. T. Stadler, J. Mollion, M.-C. Verdus, Y. Karamanos, H. Morvan, and D. Christiaen, eds., *Algal Biotechnology*, Elsevier Applied Science Publishers, Ltd., London, 1988.
11. M. A. Borowitzka and L. J. Borowitzka, *Micro-Algal Biotechnology*, Cambridge University Press, Cambridge, U.K., 1988.
12. L. Telek and H. D. Graham, eds., *Leaf Protein Concentrates*, Avi Publishing Co., Westport, Conn., 1983.
13. D. K. Salunkhe, J. V. Chavan, R. N. Adsule, and S. S. Kadam, eds., *World Oilseeds: Chemistry, Technology and Utilization*, Van Nostrand Reinhold Co., Inc., New York, 1992.
14. F. H. Steinke, D. H. Waggle, and M. N. Volgarev, eds., *New Protein Foods in Human Health*, CRC Press, Boca Raton, Fla., 1992.
15. R. E. Martin and R. L. Cullette, eds., *Engineered Seafood Including Surimi*, Noyes Data Corp., Park Ridge, N.J., 1990.
16. T. C. Lanier and C. M. Lee, eds., *Surimi Technology*, Marcell Dekker, Inc., New York, 1992.
17. A. M. Pearson and T. R. Dutson, eds., *Edible Meat By-Products: Advances in Meat Research*, Vol. **5**, Elsevier Science Publishing Co., Inc., New York, 1989.
18. J. E. Kinsella and W. G. Soucie, eds., *Food Proteins*, The American Oil Chemists Society, Champaign, Ill., 1990.
19. F. V. Kosikowski, *Cheese and Fermented Milk Foods*, 2nd ed., F. V. Kosikowski and Associates, Brooktondale, N.Y., 1982.
20. J. H. Litchfield, *Science* **219**, 740 (1983).
21. J. H. Litchfield, *Chem. Tech.* **8**, 218 (1978).
22. S. C. Prescott and C. G. Dunn, *Industrial Microbiology*, 3rd ed., McGraw-Hill Book Co., Inc., New York, 1959, Chapt. 3.
23. C. J. Soeder in Ref. 9, 9–19.
24. A. Vonshak and A. Richmond, *Biomass*, **15**, 233 (1988).
25. J. R. Benemann, *Develop. Ind. Microbiol.* **31**, 247 (1990).
26. R. A. Kay, *Crit. Rev. Food Sci. Nutr.* **30**, 555 (1991).
27. A. Klausner, *Bio/Technology* **4**, 947 (1986).
28. D. D. Duxbury, *Food Proc.* **50**(12), 50 (1989).
29. M. Javanmardian and B. O. Palsson, *Biotechnol. Bioeng.* **38**, 1182 (1991).
30. M. Oguchi, K. Otsubo, K. Nitta, A. Shimada, S. Fujil, T. Koyano, and K. Miki in R. D. MacElroy, B. G. Thompson, T. W. Tibbitts, and T. Volk, eds., *Controlled Ecological Life Support Systems, Natural and Artificial Ecosystems*, NASA Conf. Pub. 10040, National Aeronautics and Space Administration, Ames Research Center, Moffett Field, Calif., 1989, 165–173.
31. C. J. Soeder and E. Hegewald in Ref. 10, p. 68.
32. F. Comacho Rubio, M. E. Martinez Sancho, S. Sanchez Villasclaras, and A. Delgado Perez, *Process Biochem.*, **24**(4), 133 (1989).
33. P. Soong in Ref. 9, 97–113.
34. K. Kawaguchi in Ref. 9, 25–33.
35. Y.-K. Lee and C.-S. Low, *Biotechnol. Bioeng.* **38**, 995 (1991).
36. L. V. Venkataraman, B. P. Nigam, and P. K. Ramanathan in Ref. 9, 81–95.

37. H. Durand-Chastel in Ref. 9, 51–64.
38. H. Durand-Chastel and G. Clement in A. Chavez, H. Bourges, and S. Basta, eds., *Proceedings of the 9th International Congress on Nutrition*, Vol. **3**, S. Karger, Basel, Switzerland, 1975, 84–90.
39. A. Vonshak, A. Abeliovich, S. Boussiba, S. Arad, and A. Richmond, *Biomass* **2**, 175 (1982).
40. M. Kobayashi and S. I. Kutara, *Process Biochem.* **13**(9), 27 (1978).
41. M. Kobayashi, M. Kubayashi, and H. Nakanishi, *J. Ferment. Technol.* **49**, 817 (1971).
42. K. Driessens, L. Liessens, S. Masduki, W. Verstraele, H. Nelis, and A. de Leeheer, *Process Biochem.* **22**(6), 160 (1987).
43. R. H. Shipman, I. C. Kao, and L. T. Fan, *Biotechnol. Bioeng.* **17**, 1561 (1975).
44. F. H. Mohn in Ref. 9, 547–571.
45. J. Benemann, B. Koopman, J. Weissman, D. Eisenberg, and R. Goebel in Ref. 9, 457–495.
46. J. de la Noue and N. de Pauw, *Biotech. Adv.* **6**, 725 (1988).
47. *Earthrise Newsletter No. 12.* Earthrise Company, San Rafael, Calif., 1988, 2 pp.
48. E. F. McFarren, M. L. Schafer, J. E. Campbell, K. H. Lewis, E. T. Jensen, and E. J. Schantz, *Adv. Food Res.* **10**, 135 (1960).
49. G. Clement in S. R. Tannenbaum and D. I. C. Wang, eds., *Single Cell Protein II*, MIT Press, Cambridge, Mass., 1975, 467–474.
50. J. H. Litchfield, *Bioscience* **30**, 387 (1980).
51. P. Tapie and A. Bernard, *Biotechnol. Bioeng.* **32**, 873 (1988).
52. O. P. Walz and H. Brune in Ref. 9, 733–744.
53. M. Anusuya Devi and L. V. Venkataraman, *J. Food Sci.* **49**, 24 (1984).
54. *Food Nutrition Meeting Report*, Ser. No. 52, Food and Agricultural Organizational, World Health Organization, Rome, 1973.
55. J. A. Lubitz, *J. Food Sci.* **28**, 229 (1963).
56. B. Lipstein and S. Hurwitz in Ref. 9, 667–685.
57. D. L. R. Narasimha, G. S. Venkataraman, S. K. Duggal, and B. O. Eggum, *J. Sci. Food Agri.* **33**, 456 (1982).
58. C. I. Waslien, *Crit. Rev. Food Sci. Nutr.* **6**, 77 (1975).
59. G. A. Leveille, H. E. Sauberlich, and J. Shockley, *J. Nutr.* **76**, 423 (1962).
60. S. K. Lee, H. M. Fox, C. Kies, and R. Dam, *J. Nutr.* **92**, 281 (1967).
61. H. D. Payer, W. Pabst, and K. H. Runkel in Ref. 9, 785–797.
62. J. H. Litchfield in H. J. Peppler and D. Perlman, eds., *Microbial Technology*, 2nd ed., Vol. **2**, Academic Press, Inc., New York, 1979, 93–155.
63. J. H. Litchfield in Ref. 4, 65–109.
64. J. S. Gow, J. D. Littlehailes, S. R. L. Smith, and R. B. Walter in S. R. Tannenbaum and D. I. C. Wang, eds., *Single Cell Protein II*, MIT Press, Cambridge, Mass., 1975, 370–384.
65. D. G. MacLennan, J. S. Gow, and D. A. Stringer, *Process Biochem.* **8**(6), 22 (1973).
66. G. H. Evans in G. H. Evans *Single Cell Protein*, MIT Press, Cambridge, Mass., 1975, 243–254.
67. U.S. Pat. 3,846,238 (Nov. 5, 1974), G. H. Evans and J. L. Shennan (to British Petroleum Co.).
68. U.S. Pat. 3,865,691 (Feb. 11, 1975), J. A. Ridgeway, T. A. Lappin, B. M. Benjamin, J. B. Corns, and C. Akin (to Standard Oil Co. of Indiana).
69. N. H. Mermelstein, *Food Technol.* **43**(7), 50 (1989).
70. D. O. Hitzman in Ref. 7, 27–32.
71. S. Bernstein, G. H. Tzeng, and D. Sisson in A. E. Humphrey and E. L. Gaden, Jr., eds., *Single-Cell Protein from Renewable and Nonrenewable Resources*, John Wiley & Sons, Inc., New York, 1977, 35–44.
72. S. Bernstein and P. E. Plantz, *Food Eng.* **48**(11), 74 (1977).
73. L. K. Shay and G. H. Wagner, *J. Dairy Sci.* **69**, 676 (1986).
74. G. L. Solomons in H. W. Blanch, S. Drew, and D. I. C. Wang, eds., *Comprehensive Biotechnology*, Vol. **3**, Pergamon Press, Oxford, U.K., 1985, 483–505.
75. H. Romantschuk in Ref. 64, 344–355.
76. H. Romantschuk and M. Lehtomaki, *Process Biochem.* **13**(3), 16, 17, 23 (1978).
77. L. K. Shay and G. H. Wegner, *Food Technol.* **38**(10), 61 (1985).
78. C. A. Shacklady and E. Gatumel in H. Gounelle de Pontanel, ed., *Proteins from Hydrocarbons*, Academic Press, New York, 1973, 27–52.

79. *Type B Torula Dried Yeast*, Lake States Yeast, Rinelander, Wis., 1986.
80. *Torutein Product Bulletin*, Amoco Foods Co., Chicago, Ill., 1974.
81. M. S. Erdman, W. G. Bergen, and C. A. Reddy. *Appl. Environ. Microbiol.* **33**, 901 (1977).
82. J. H. Litchfield in Ref. 74, 463–481.
83. R. Bressani in Ref. 65, 90–121.
84. G. Sarwar, R. W. Peace, and H. G. Botting in Ref. 7, 107–116.
85. G. L. Solomons in Ref. 7, 19–26.
86. I. Duthie in Ref. 64, 505–544.
87. N. S. Scrimshaw in C. W. Robinson and J. A. Howell, eds., *Comprehensive Biotechnology*, Vol. **4**. Pergamon Press, Oxford, U.K., 1985, 673–684.
88. *CFR Title 21, Food and Drugs 172.325, 172.896*, U. S. Government Printing Office, Washington, D.C., 1992.
89. N. W. Pirie in Ref. 12, 1–9.
90. N. W. Pirie, ed., *Leaf Protein: Its Agronomy, Preparation, Quality, and Use*, IBP Handbook No. 20, Blackwell Scientific Publications, Oxford, U.K., 1971, 53–62, 86–91, 155–163.
91. L. Telek in Ref. 12, 295–395.
92. G. O. Kohler and E. M. Bickoff, in Ref. 90, 69–77.
93. G. O. Kohler, R. H. Edwards, and D. E. de Fremery in Ref. 12, 508–524.
94. L. Koch in Ref. 12, 601–632.
95. T. O. Addy, L. F. Whitney, and C. S. Chen in Ref. 12, 490–507.
96. F. Favati, J. W. King, J. P. Friedrich, and K. Eskins, *J. Food Sci.* **53**, 1532 (1988).
97. S. Nagy and H. E. Nordby in Ref. 12, 268–294.
98. G. O. Kohler, S. G. Wildman, N. A. Jorgenson, R. V. Enochian, and W. J. Bray in M. Milner, N. S. Scrimshaw, and D. I. C. Wang, eds., *Protein Resources and Technology: Status and Research Needs*, Avi Publishing Co., Westport, Conn., 1978, 543–569.
99. R. Carlsson and P. Hanczakowski, *J. Sci. Food Agr.* **36**, 946 (1985).
100. C. K. Lyon, P. F. Knowles, and G. O. Kohler, *J. Sci. Food Agr.* **34**, 849 (1983).
101. M. Byers in Ref. 12, 135–175.
102. S. J. Sheen, *J. Agric. Food Chem.* **39**, 681, (1991).
103. R. V. Enochian, G. O. Kohler, R. H. Edwards, D. D. Kuzmicky, and C. J. Vosloh, Jr. in Ref. 12, 525–545.
104. C. Savangikar and M. Ohshima, *J. Agric. Food Chem.* **35**, 82 (1987).
105. K. Sunder Rao, R. Dominic, K. Singh, C. Kaluwin, D. E. Rivett, and G. P. Jones, *J. Agric. Food Chem.* **38**, 2137 (1990).
106. J. Maciejewicz-Rys and P. Hanczakowski, *J. Sci. Food Agr.* **50**, 99 (1990).
107. N. Singh, in Ref. 91, 131–134.
108. R. P. Devedas and N. K. Murthy, *World Rev. Nutr. Diet* **31**, 159 (1978).
109. B. E. Knuckles and G. O. Kohler, *J. Agr. Food Chem.* **30**, 748 (1982).
110. W. E. Barbeau and J. E. Kinsella, *J. Food Sci.* **52**, 1030 (1987).
111. S. J. Trank, D. W. Johnson, and E. L. Cussler, *Food Technol.* **43**(6), 78 (1989).
112. L. R. Dahl and R. Villota, *J. Food Sci.* **56**, 1002 (1991).
113. A. M. Pearson, *Bioscience* **26**, 249 (1976).
114. J. E. Kinsella, *J. Am. Oil Chem. Soc.* **56**, 242 (1979).
115. G. MacLeod and J. Ames, *Crit. Rev. Food Sci. Nutri.* **27**, 219 (1988).
116. W. H. Martinez, *J. Am. Oil Chem. Soc.* **56**, 280 (1979).
117. J. J. Spadaro and H. K. Gardner, Jr., *J. Am. Oil Chem., Soc.* **56**, 422 (1979).
118. Y-M Tzeng, L-L Diosady, and L. J. Rubin, *J. Food Sci.* **55**, 1147 (1990).
119. K. D. Schwenke, J. Kroll, R. Lange, M. Kujawa, and W. Schnack, *J. Sci. Food Agr.* **51**, 391 (1990).
120. S. M. Claughton and R. J. Pearce, *J. Food Sci.* **54**, 357 (1989).
121. R. Tasneem and V. Prakash, *J. Sci. Food Agr.* **59**, 237 (1992).
122. C.-Y. Ma and G. Khanzada, *J. Food Sci.* **52**, 1583 (1987).
123. T. V. Hung, P. D. Hanson, V. C. Amenta, W. S. A. Kyle, and R. S. T. Yu, *J. Sci. Food Agr.* **41**, 131 (1987).
124. L. A. Bryant, J. Montecalvo, Jr., K. S. Murey, and B. Loy, *J. Food Sci.* **53**, 810 (1988).
125. C. S. Lin, and J. F. Zayas, *J. Food Sci.* **52**, 1308 (1987).
126. C-S Lin and J. F. Zayas, *J. Food Sci.* **52**, 1615 (1987).

127. P. Yue, N. Hettiarachchy, and B. L. D'Appolonia, *J. Food Sci.* **56**, 992 (1991).
128. R. Ohlson and K. Anjou, *J. Am. Oil Chem. Soc.* **56**, 431 (1979).
129. A. I. Ihekoronye, *J. Sci. Food Agr.* **54**, 89 (1991).
130. A. T. Paulson and M. A. Tung, *J. Food Sci.* **52**, 1557 (1987).
131. A. T. Paulson and M. A. Tung, *J. Food Sci.* **53**, 817 (1988).
132. R. Ponnampalam, M. A. Vijayalakshmi, L. Lemieux, and J. Amiot, *J. Food Sci.* **52**, 1552 (1987).
133. F. F. Shih, *J. Food Sci.* **52**, 1529 (1987).
134. F. F. Shih, *J. Food Sci.* **56**, 452 (1991).
135. J. S. Hamada, *J. Food Sci.* **56**, 1725 (1991).
136. J. Velisek, T. Davidek, V. Kubel, and I. Viden, *J. Food Sci.* **56**, 139 (1991).
137. *CFR Title 21, Food and Drugs 155.170, 155.201, 161.190*, U. S. Government Printing Office, Washington, D.C., 1992.
138. *The Food Chemical News Guide*, 228.1 (Aug. 17, 1992).
139. *CFR Title 21, Foods and Drugs, 172.340, 172.385*, U. S. Government Printing Office, Washington, D.C., 1992.
140. V. D. Sidwell, B. R. Stillings, and G. M. Knobl., Jr., *Food Technol.* **24**, 876 (1970).
141. C. Cheftel, M. Phern, D. I. C. Wang, and S. R. Tannenbaum, *J. Agr. Food Chem.* **19**, 155 (1971).
142. R. Miller and H. S. Groninger, Jr., *J. Food Sci.* **41**, 268 (1976).
143. C. M. Lee, *Food Technol.* **40**(3), 115 (1986).
144. G. A. MacDonald and T. Lanier, *Food Technol.* **45**(3), 150 (1991).
145. K. H. Chung and C. M. Lee, *J. Food Sci.* **56**, 263 (1991).
146. G. B. Quaglia and E. Orban, *J. Food Sci.* **55**, 1571 (1990).
147. B. D. Rebeca, M. T. Pena-Vera, and M. Diaz-Castaneda, *J. Food Sci.* **56**, 309 (1991).
148. S. Eriksen and I. S. Fagerson, *J. Food Sci.* **41**, 490 (1976).
149. M. Fujimaki, H. Kato, S. Arai, and M. Yamashita, *J. Appl. Bacteriol.* **34**, 119 (1971).
150. M. Yamashita, S. Arai, and M. Fujimaki, *J. Food Sci.* **41**, 1029 (1976).
151. J. Montecalvo, Jr., S. M. Constantinides, and C. S. T. Yang, *J. Food Sci.* **49**, 1305 (1984).
152. M. R. Raghunath and A. R. McCurdy, *J. Sci. Food Agric.* **54**, 655 (1991).
153. M. Yamashita, S. Ari, S. J. Tsai, and M. Fujimaki, *J. Agr. Food Chem.* **19**, 1151 (1971).
154. S. W. Fox, J. W. Frankenfeld, D. Romsos, B. M. Robinson, and S. A. Miller in Ref. 98, 569–583.
155. *PAG Bulletin, Policy Statement No. 4, Single-Cell Protein*, 1970; *Guidelines No. 6, Preclinical Testing of Novel Sources of Protein*, 1970; *No. 7, Human Testing of Supplementary Food Mixtures*, 1970; *No. 8. Protein-Rich Mixtures for Use as Supplementary Foods*, 1971; *No. 12, Production of Single Cell Protein for Human Consumption*, 1972; *No. 15, Nutritional and Safety Aspects of Novel Protein Sources for Animal Feeding*, 1974; Protein Advisory Group of the United Nations, FAO/WHO/UNICEF, United Nations, New York.

JOHN H. LITCHFIELD
Battelle Memorial Institute

Related Articles

Food processing; Feeds and feed additives; Microbial transformation