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Whey, a Potential Substrate for Biotechnology

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Introduction

COMPOSITION

Whey is a by-product of the cheese-making industry following the separation of casein and butter fat as curd from milk. Whey composition varies according to its origin (ewe, goat or cow) and to the cheese-making technique employed. The composition of whey has been widely studied (Supplee, 1940; Olling, 1963; Guy, Vettel and Pallanche, 1966; Kosikowski, 1967; Mereo, 1971, Cerbulis, Woychik and Wodolowski, 1972; Mavropoulou and Kosikowski, 1973a, b; Kosikowski, 1975; Février and Bourdin, 1977). Cheese wheys can be divided into five groups (*Table 1*). The coagulation with rennet or rennet preparations yields sweet whey (pH 4·5–6·7), with high lipid contents. Coagulation by lactic

Table 1. Composition of different types of liquid whey (g/ℓ) according to Février and Bourdin. 1977).

	Cow			Ewe	Goat
Curd type	Rennet	Mixed	Lactic	Rennet	Lactic
Density	1·239	1·0247	1·0245	1·0234	1·0269
Dry matter	70·84	70·49	65·76	83·84	62·91
Lipids	5·06	3·38	0·85	6·46	0·40
Dry matter without lipids	65·78	67·11	64·91	77·38	62·51
Lactose	51·81	50·84	45·25	50·98	39·18
Total nitrogen	1·448	1·454	1·223	2·933	1·466
Non-protein nitrogen	0·368	0·414	0·536	0·796	0·669
Ammonium nitrogen	0·041	0·090	0·140	0·129	0·176
Urea nitrogen	0·141	0·095	0·070	0·139	0·122
Lactic acid	0·322	2·226	7·555	1·763	8·676
Citric acid	1·298	1·095	0·260	1·032	0·157
Ash	5·252	5·888	7·333	5·654	8·361
Phosphorus	0·412	0·470	0·649	0·545	0·703
Calcium	0·466	0·630	1·251	0·494	1·345
Potassium	1·455	1·491	1·485	1·281	1·812
Sodium	0·505	0·537	0·528	0·616	0·433
Chlorides (as NaCl)	2·195	2·208	2·092	2·368	3·287

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Table 2. Average composition of defatted whey (% of dry matter) (according to Février and Bourdin, 1977).

	Cow			Ewe	Goat
Curd type	Rennet	Mixed	Lactic	Rennet	Lactic
Dry defatted extract	100	100	100	100	100
Lactose	78·76	75·76	69·71	65·88	62·68
Total nitrogen	2·20	2·17	1·88	3·79	2·35
Total protein (N × 6·38)	14·04	13·82	12·02	24·18	14·96
Total protein (N × 6·25)	13·75	13·56	11·75	23·69	14·69
Non-protein nitrogen	0·56	0·62	0·83	1·03	1·07
Ammonium nitrogen	0·06	0·13	0·22	0·17	0·28
Urea nitrogen	0·21	0·14	0·11	0·18	0·20
Lactic acid	0·49	3·32	11·64	2·28	13·38
Citric acid	1·97	1·63	0·40	1·33	0·25
Ash	7·98	8·77	11·30	7·31	13·37
Calcium	0·71	0·94	1·93	0·64	2·15
Phosphorus	0·63	0·70	1·00	0·70	1·12
Potassium	2·21	2·22	2·29	1·66	2·90
Sodium	0·77	0·80	0·81	0·80	0·69
Chlorides (as NaCl)	3·34	3·29	3·22	3·06	5·26

fermentation yields acid whey (pH 3·9–4·5), containing smaller quantities of lactose and proteins. It is noteworthy that whey obtained from ewe's milk contains more than twice the amount of protein found in whey from cow's milk. The comparison between the content of non-fat solids of wheys of different origins (*Table 2*), proposed by Février and Bourdin (1977), shows the importance of two factors: the high content of lactose (75%), and that of protein (N × 6·25) which varies from 12% to 14% of dry matter. Cheese whey and ultrafiltration permeate contain trace elements (*Table 3*) and the main vitamins (*Table 4*): whey is therefore extremely valuable nutritionally, and the permeates can be used for the industrial production of many micro-organisms capable of using lactose or its hydrolysis products.

QUANTITIES AVAILABLE

According to the Whey Product Institute of Chicago, Illinois, 11 804 000 t of whey were produced in the US in 1974 and 16 355 000 t were produced in 1979. In France, whey production rose from 4 800 000 t in 1974 to 8 000 000 t in 1979, and still continues to increase (SCEES, 1981). According to Coton (1983), world whey production had reached 118 261 000 t by 1981.

Initially, whey was considered to be a waste product to be disposed of, and was mainly redistributed to milk producers for animal feed. The increase in size of cheese plants, the necessity for reduction in the BOD and COD of the effluent (*see* Chapter 10, this volume) and the need to maximize returns on raw material have encouraged producers to seek new ways of using cheese whey. At first, cheese whey was concentrated or dried for animal feed. Since 1960, great efforts have been made to fractionate and use independently the main

Table 3. Average content of the main trace elements in cheese whey and ultrafiltration permeate (mg/100 g dry matter).

Element	Whey	Permeate
Iron	1–7	3–11
Copper	0·5–5	1–3
Zinc	5–9	30–33
Manganese	0·01–0·04	0·5–0·8

Table 4. Average vitamin content of cheese whey and ultrafiltration permeate (mg/100 g of dry matter).

Vitamin	Whey	Permeate
Vitamin A	100	80
Thiamin	4–6	5–6
Pyridoxin	6–10	5–10
Riboflavin	7–30	15–20
Calcium pantothenate	30–70	50–60
Biotin	0·2–0·3	0·1–0·3
Cobalamin	0·01–0·05	0·02–0·05
Vitamin C	30–50	20–40

constituents of whey, in particular whey proteins. Despite these efforts, only about 50% of world whey production has been turned to good account.

Utilization of cheese whey

LIQUID CHEESE WHEY

The use of liquid cheese whey for the purpose of feeding hogs is on the decline because of the constant increase in the output from cheese plants. Such large volumes are difficult to redistribute to farmers and would require the establishment of large feedlots nearby. However, in the case of small cheese plants, Février and Bourdin (1977) showed that this use remained of interest. Similarly, it is possible to incorporate into cattle and sheep feed rations up to 30% of dry matter as liquid whey (Schingoethe, 1976).

With regard to human food uses, the production of beverages from cheese whey has been attempted in several countries. Holsinger, Posati and De Vilbiss (1974), Holsinger *et al.* (1977) and Kosikowski (1981) reviewed different whey beverage processes. Several products, either protein-enriched or deproteinized, and either fermented or non-fermented, were tested. However, it is noteworthy that only the Rivella product has been marketed in Europe. A lactic fermentation was used for the production of this beverage.

CONCENTRATED CHEESE WHEY

The concentration processes for cheese whey were developed in order to reduce the costs of transport and storage and to improve the quality of the product

(Longuet, 1977). The technologies that were developed employed either the reverse osmosis technique (Pepper, 1981) or the multiple evaporation effect, with or without mechanical recompression of steam (Voisin, 1981). The reverse osmosis technique is well adapted to small production units whereas the concentration process through evaporation would require larger installations. Whey has sometimes been concentrated to 18–38% in order to facilitate transport. Concentrates at 40% and 60% could be used for animal feed. Molasses at 70% dry matter and cattle lick containing 75% dry matter have also been used in animal rations (Coton, 1980). Thivend, Vermorel, and Guilhermet (1977) showed that the use of whey concentrates was well adapted to cattle feeding. These authors were convinced that deproteinized permeate could also be used. Nitrogen could be provided by non-protein additives. Similarly, Barre *et al.* (1977) showed that concentration of whey yielded a microbiologically stable product at a lower production cost than that of drying.

WHEY POWDER

Although the production of whey powder is expensive and requires large amounts of energy, it is still a greatly used process because whey powder is easy to store and to transport. There is often a pre-concentration process by evaporation down to 50% dry matter, followed by drying in a spray tower (Chaput, 1977; Longuet, 1977; Hynd, 1980).

With regard to animal feed, whey powder is extensively used in hog rations and also in milk replacers for unweaned calves. This outlet is very important in Europe, and especially in France where the production of whey powder reached 285 000 t in 1979 (Lenoir, 1981). According to Toullec and Le Treut (1977) about 100 000 t of whey powder were incorporated into unweaned calves' rations in 1975 in France. However, it is noteworthy that present European legislation requires the incorporation (up to 60%) of skim milk powder into these rations: the development of this market for whey powder is thus becoming limited and unstable.

Many studies on the incorporation of whey powder into human foods (Guy, Wettel and Pallanche, 1966; de la Guérivière, 1977; Kosikowski, 1979; Mathur and Shahani, 1979; Grandadam, 1981; Mann, 1982) have shown that sweet whey can be added advantageously to many foods such as frozen desserts, cheese products, dried soup and gravy bases.

The main products containing whey powder have been described by Kosikowski (1979), Clark (1979), and Salmon (1981). The main industries using whey are infant food manufacturers, bakers, confectioners and meat processing plants. The amounts used vary very much from one country to another. Kosikowski (1979) estimated that about 30% of whey powder was incorporated into food products in the USA, whereas only about 1% was so incorporated in the UK.

The intended use of whey powder depends as much on its functional properties as on its nutritional value. These functional properties depend largely on the different treatments applied before the drying stage. Whey can be used as it is, partly or totally demineralized by electrodialysis or by ion exchange, deproteinized by ultrafiltration, or both demineralized and deproteinized. In fact,

all these technologies have been employed in order to obtain a product with the best functional properties.

Protein recovery

From the nutritional point of view, cheese whey is unbalanced by its high mineral and lactose contents. Lactose is not well assimilated by many organisms. This had led to the early development of many processes for the recovery of proteins which constituted the most valuable part of whey. Recently, the ultrafiltration technique has allowed the retention of almost all milk proteins and has increased the yield in cheese manufacture (Maubois, Mocquot and Vassal, 1969). As a result it is now the permeates which need to be turned to better account.

PROTEIN PRECIPITATION PROCESSES

There are several cheap and easy processes for the recovery of proteins through precipitation at their isoelectric pH and at 90–95°C (Centriwhey, Bel Industrie). The industrial uses of these processes are, however, limited by the partial or total denaturation of the proteins thus obtained.

Moddler and Harwalkar (1981) proposed different heat treatments at acid pH (pH 2·5–3·5) at 95°C. The proteins thus obtained had interesting functional properties (Moddler and Emmons, 1977). Similarly, heat treatment at neutral pH (pH 6·8–7·5 at 80°C) increased the foaming power of the proteins obtained (Dewitt and Hontelez-Backx, 1981). Cold precipitation with salts, polymers or solvents has been reviewed recently by Mathur and Shahani (1979) and by Humbert and Alais (1981a). The main precipitation agents used were carboxymethyl cellulose, polyphosphates, ferriphosphate, polyacrylic acid, polyethylene glycol, chitosan, bentonite, lignosulphate and sodium laurylsulphate. Protein yields varied from 63% with polyacrylic acid to 91% for ferripolyphosphate. The precipitate contained a high proportion of ash, requiring further demineralization.

ULTRAFILTRATION

Ultrafiltration for the recovery of proteins was proposed in 1969 by Fallick, and this technology was rapidly developed by the dairy industry. According to Maubois and Brulé (1982), the area of filter membrane installed has increased from 300 m^2 in 1971 to 70 000 m^2 in 1981. In 1979, about 3% of available world whey production employed this technique, which reached 8–10% in some countries that are major producers of cheese.

Maubois (1980) and Maubois and Brulé (1982) have described the main types of membranes being used: flat sheet (proposed by Rhône-Poulenc, Pasilac and Dorr-Oliver), tube shape (by SFEC, Abcor, PCI, Wafilin), hollow fibre (by Romicon), spiral shape (by Abcor). According to Maubois (1980), the first-generation membrane made of cellulose acetate has now almost entirely been superseded. The membranes currently used are manufactured from synthetic organic polymers. These second-generation membranes have a better performance,

and a better resistance to extremes of pH and temperature. More recently, mineral membranes (zirconium oxide on carbon graphite) have appeared with very high mechanical (2000 kPa), thermal (400°C) and physico-chemical resistances. These new membranes are of great interest for the ultrafiltration of solutions of very high protein content (Maubois, 1979; Goudefranche *et al* 1981).

The protein concentrates obtained by ultrafiltration have particularly interesting nutritional (Forsum, 1974) and functional properties (Dewitt and de Boer, 1975; Cheftel and Lorient, 1982), and could be incorporated into soft curd cheese. The protein concentrates are also useful animal-feed components, especially for unweaned calves (Stewart, Muller and Griffin, 1974; Toullec *et al.*, 1975). Their functional properties (solubility, viscosity, water-holding capacity, emulsifying power, foaming power) have encouraged attempts to use them in a great number of food products in replacing other more traditional additives such as milk powder and egg albumen (Richert, 1979; Paquet, 1981). The incorporation of these protein concentrates into new products such as gel-type foods (Humbert and Alais, 1981b) and comminuted meat products (Grandadam, 1981) has also been investigated.

The direct ultrafiltration of milk for preparing liquid 'pre-cheese' has been described by Maubois, Mocquot and Vassal (1969) (MMV process). This cheese-making technology produces, instead of whey, a deproteinized permeate which needs further processing. In 1981, 120000 t of cheese were produced applying the ultrafiltration technique (Maubois and Brulé, 1982). Feta cheese production has been a major development in Denmark. Recent improvement in ultrafiltration equipment and, subsequently, in product quality (Mahaut *et al.*, 1982), should soon allow this technique to be used in the production of soft curd cheese.

ION-EXCHANGE SEPARATION

Two processes are being tested. The industrial process Vistec uses carboxymethyl cellulose to fix the proteins at pH 4, with elution at pH 9. The eluate has to be concentrated by ultrafiltration. The foaming property of the product obtained is similar to that of egg white (Palmer, 1977). The spherosil process (Mirabel 1978 a,b; 1981) uses controlled silica beads of a predetermined uniform size on which anionic or cationic active groups have previously been bonded. The protein retention capacity can reach 130 g/kg support. For treating acid whey, the process uses cation exchange beads at pH 4·6 whereas for treating sweet whey the column is filled with anion exchange beads at pH 6·6. The protein content of the eluate is 4·5%. This process leads to the production of undenatured, very pure proteins which can be separated from each other.

These processes may be used eventually for large-scale fractionation of proteins: in particular, it should be possible to produce immunoglobulins.

PROTEIN HYDROLYSATES

The production of protein hydrolysates with a membrane enzyme reactor was suggested by Roger, Brulé and Maubois (1981). This process comprised three stages: the preparation of a protein concentrate (70–90 g protein/ℓ); clarification

of the concentrate, and hydrolysis in an enzyme reactor. The hydrolysis could be performed with a multienzyme system such as proteases or lipases, or with a single purified enzyme. This reactor functioned in the continuous state. When the protein concentrate was hydrolysed by the reactor with the enzyme pancreatin, Roger (1979) and Roger and Maubois (1981) showed that the hydrolysates obtained could have satisfied the nutritional nitrogen needs of post-operative patients. Using the same technique, Brulé *et al.* (1980) proposed the hydrolysis of a caseinate solution followed by the formation of phosphopeptidic complexes by the addition of calcium chloride and disodium phosphate; these phosphopeptide complexes were subsequently separated and purified. Phosphopeptides are potentially of interest because of their effect on the intestinal absorption of minerals (Mathan *et al.*, 1979; Mykkanen and Wasserman, 1980).

Utilization of permeates

All the processes described in the previous section yield a permeate with high contents of lactose (35–50 g/ℓ), minerals, vitamins, and sometimes lactic acid. The volume of permeates being produced is increasing. As there are several profitable uses at the preceding production step, either by incorporation of the proteins into cheese products or by recovery and fractionation of these proteins, the permeates have theoretically little or no value. However, the volumes produced are so large that some treatment at least is necessary before their disposal as effluent. Coton (1980) has shown that the permeate could be used *per se* or in a concentrated form for hog and cattle feed. There are also a few uses in the food industry; however, the enormous volumes of permeate to be treated demand other possible outlets, implying the use of fermentation techniques.

PRODUCTION OF FOOD YEAST

The nutritional qualities of yeasts are well recognized (Wasserman, 1961; Birolaud, 1971; Vrignaud, 1971). These qualities are related to their amino acid, sterol, fatty acid and vitamin composition. More recently the functional properties of yeast have been investigated, showing a high binding power, high water-retention capacity and a thickening power that was unaffected by heat treatment (Marzolf, 1977, 1981; de la Guérivière, 1981). The application of these functional properties will be linked with the development of new outlets in the food industry and with the use of yeast components in the pharmaceutical industry.

In a general review of food yeast, Meyrath and Bayer (1979) remarked that the production of yeast from whey was attempted as early as 1940 by Messrs Harmer in Spillern. Many other processes were subsequently proposed, such as the Polyvit process (Waeser, 1944), the Waldhoff process (Demmler, 1950), the Bel process (1958), and the Wheat process (Robe, 1964). Bel started its industrial process in 1958. The fermenters used corresponded to the air-lift-type Lefrançois–Marillier process (Lefrançois, 1964). The fermenters have not much changed to the present day: however, the biological aspects of the fermentations are better understood. Studies of the flora being used helped greatly to improve

the performance of the strains. At present there are two possibilities: transformation of lactose thus making it available to the yeast strain chosen, or direct use of lactose by a yeast strain able to metabolize this substrate.

Among the first type of process was that proposed by Lembke and Baader (1975). Lactic bacteria were used to transform lactose into lactic acid, without aeration at pH 4·5 and 44°C. In a second step, lactic acid was used by *Candida utilis* and *Candida krusei* in a vigorously aerated fermenter. This technology involved the use of two successive fermenters employing different reaction conditions, and another drawback was that the medium was at a very vulnerable pH for part of the process. The process as a whole could be run in the semi-continuous or continuous fashion (Moebus and Kiesbye, 1975). Experiments with *Lactobacillus bulgaricus* and *Candida krusei* mixed cultures showed that it was possible to attain productivities of up to 2·65 g/ℓ/h under these conditions of continuous fermentation.

The direct transformation of lactose could be achieved by several yeast strains. The strain most often used has been *Kluyveromyces fragilis* (Porges, Pepinski and Jasewicz, 1951; Wasserman, 1960; Chapman, 1966; Admunson, 1967; Ladet *et al.*, 1972; Bernstein and Plantz, 1977; Moulin and Galzy, 1976; Moulin, Ratomahenina and Galzy, 1976; Giec and Kosikowski, 1982). *Candida intermedia* was also recommended by Meyrath and Bayer (1979) for the Vienna UKM process. The final choice depends as much on the strain as on the species, because many biological factors of great economic importance vary considerably within each species, for example phosphate requirements (Wasserman, 1961; Nour-el-Dien, Halasz and Lengyel, 1981) and vitamin requirements (Wasserman, 1961, Nour-el-Dien and Halasz, 1982a,b).

Studies with industrial-scale fermenters (Moulin and Malige, unpublished data) have shown that a progressive improvement of the strains was seen for the following factors: increase in growth rate; increase in protein and nucleic acid content; decrease in the relative content of storage compounds, especially glycogen; loss of sporulation and sexual characteristics. Within the fermenter the selection operated in favour of the features useful for survival in the absence of cell multiplication. We observed the same situation in other industrial systems using a continuous fermentation technique. It is possible to select yeast strains with rapid growth rate on permeates without any additive other than a source of nitrogen; however, industrial producers generally add small amounts of trace elements (Fe, Cu, Mn, Zn). Sterilization of the medium is not necessary because of the bacterial nature of the ultrafiltration permeates and the pH usually employed (pH 3·2). Oxygen transfer remains the limiting factor for the productivity of the fermenters. There are at present two processes for the production of food yeast by direct transformation of lactose in continuous culture—the Vienna process and the Bel process.

The Vienna process

The flow diagram of this process has been given by Meyrath and Bayer (1979). The fermentation was run continuously at 32–33°C and pH 3·4–3·6, with the addition of ammonia (0·8 kg/m^3) and ammonium sulphate (0·8 kg/m^3) as nitrogen

source and for pH regulation. Under these conditions, for a lactose concentration input of 45 g/ℓ, the productivity obtained was 4·5 g/ℓ/h (yeast dry matter). Yield of dry matter for the amount of lactose used was 54%. The fermentation took place in a fermenter with an aeration system involving an external pump for rapid circulation of the reaction mixture; about 600 m^3/h of air were used for a 14 m^3 fermenter. The strain used was *Candida intermedia* which is characterized by an exclusively oxidative lactose metabolism (Lodder, 1970). This property enables undiluted permeate to be used because no alcoholic fermentation of lactose would be expected. However, this physiological advantage of the strain cannot, as yet, be exploited fully because of the limitations of the industrial fermenters currently available. The level of oxygen transfer obtained is not sufficient for the dilution rates which might be expected according to the strain and the amount of lactose used (45g/ℓ). Under laboratory conditions, the growth rate (expressed as $\mu = \log_2/T_g$, where T_g is the generation time of the yeast *Candida intermedia*) of this strain was 0·28/h. Such a growth rate could be reached in an industrial fermenter only by using diluted permeate.

The Bel process

The general scheme of this process has been described by Marzolf (1977, 1981), by de la Guérivière (1981) and by Moulin, Malige and Galzy (1983). This process is being employed, in France, in three industrial units producing about 10 000 t of food yeast annually. The fermentation has been run at pH 3·2 and 38°C ±1°C using as substrate deproteinized whey either from protein recovery units by ultrafiltration, or by protein precipitation at the isoelectric point (pH 4·5) at 90°C; in both cases the whey was stored before fermentation. Nutrients (nitrogen, trace elements) were added and the substrate was diluted before it was pumped into the fermenter. The whey solution thus prepared contained 20–25 g/ℓ of lactose and was continuously pumped into the fermentation tower; the dilution rate was constant at 0·33/h. Aeration was ensured with an air-lift system according to the scheme proposed by Lefrançois (1964) at 1·5 vol/vol/min. For a fermenter of 23 m^3 capacity, about 1800 m^3air was pumped per hour. Such a fermenter would produce 4·5 g yeast dry matter per litre per hour (55–60 g yeast per 100 g lactose utilized). Unlike the Vienna process, which uses a pure strain, the Bel process uses three species in equilibrium: Moulin, Malige and Galzy (1983) have shown that this equilibrium is between strains of the species *Kluyveromyces fragilis*, *Kluyveromyces lactis* and *Torulopsis bovina*, with *Kluyveromyces fragilis* strains representing 90% of the total flora. These strains grow well on lactose: they possess the general physiological feature of lack of glucose effect on their respiratory metabolism and a strong Pasteur effect (Chassang-Douillet *et al.*, 1973): they could, therefore, grow on a sugar substrate in the presence of air with a very weak fermentative metabolism. When oxygen was not the limiting factor, the production of ethanol was 0·06 mg/h per gram of yeast dry matter (Moulin, Legrand and Galzy, 1983). This ethanol production was sufficient to maintain the strains of *Torulopsis bovina* strains in the fermenter (Moulin, Malige and Galzy, 1983) (this species grows on ethanol but not on lactose); the strains of *Kluyveromyces lactis* species grew on lactic acid.

The equilibrium between the strains of these three species was remarkably stable. In fact, each species had an independent 'ecological niche' because each of them preferentially uses a different substrate. This equilibrium of the flora enables the various types of permeate to be treated under optimal conditions. The relative proportions of the three species may vary according to the relative lactose and lactic acid contents of the permeates, the lactose content of the fermenter being the most important factor. When this lactose input increased from 20 to 27 g/ℓ, the dry-matter yield was shown to fall from 63% to 48% (Moulin, Malige and Galzy, 1983). Again, in this process the limiting factor was oxygen transfer. It is noteworthy that the productivity of these two industrial processes was identical despite the technological differences and the different yeast strains used.

PRODUCTION OF PROTEIN-ENRICHED WHEY

In the studies described above, the aim was to produce yeast cells with separation of the micro-organisms from the medium. There have also been several studies on the production of a protein-enriched food, exploiting the growth of micro-organisms (yeast, filamentous fungi). At present, only the first two processes described below are used commercially.

The Devos process consists of growing *Saccharomyces cerevisiae* Hansen strain on the lactic acid in whey; the whey thus enriched is concentrated and dried. The yeast-enriched powder contains 15–18% protein.

The Société des Alcools du Vexin (SAV) process (1963) involves a two-stage fermentation followed by evaporation and drying. The substrate in the first fermenter is lactose, and lactic acid is used in the second fermenter. The productivity is 2 g/ℓ/h with a dry-matter yield from lactose of between 36% and 41%. This process can utilize whey from cheese plants or from casein plants as substrate.

The production of yeast-enriched milk proteins has also been studied by Molinaro, Hondermarck and Jacquot (1977). The principle of this process consisted of growing a *Kluyveromyces fragilis* strain on cheese whey. The whole culture mixture is subsequently concentrated or dried: the resulting powder contains up to 45% proteins (N × 6·25).

The enrichment of whey proteins has also been attempted using yeast-like moulds. Halter, Puhan and Kapelli (1981) proposed the enrichment of ultrafiltration permeates with a strain of *Trichosporon cutaneum*. The experiments performed with equipment of capacity 5000–7000 ℓ used a fed-batch culture technique. The ultrafiltration permeate with 46 g/ℓ lactose was first enriched with nitrogen (NH_4Cl 6·0 g/ℓ), trace elements and vitamins. Cell growth was limited by the restricted addition of ammonia so that only about 50% of lactose was consumed. The fermentation was run at pH 4 following pasteurization of the medium in order to avoid contamination by wild yeasts. The final product thus obtained contained 1·3–1·4% protein and 1·3–2% lactose as dry matter.

The Caliqua Sireb process (Fleury and Henriet, 1977) used a filamentous fungus of the species *Penicillium cyclopium* in a continuous culture. The medium contained amino acids and soluble peptides at the outlet of the fermenter. The

biomass obtained could be dried and the protein content (N × 6·25) of the final product was about 40%.

PRODUCTION BY BACTERIAL FERMENTATION, OF A RUMINANT FEED SUPPLEMENT RICH IN CRUDE PROTEIN

Different processes have been described for the fermentation of lactose from whey into lactic acid (Whittier and Rogers, 1931; Johnson *et al.*, 1937; Jansen, 1945). Many authors have shown that ammonium salts of short-chain organic acids, in particular ammonium lactate, are better nitrogen sources for ruminants than urea or soybean meal (Varner and Woods, 1971; Allon and Henderson, 1972; Dutrow, Huber and Henderson, 1974; Crickenberger, Henderson and Reddy, 1981). The fermentation could be batch (Gerhardt and Reddy, 1978; Juengtst, 1979) or continuous (Keller and Gerhardt, 1975; Reddy, Henderson and Erdman, 1976), or continuous with dialysis (Coulman, Stierber and Gerhardt, 1977; Stierber and Gerhardt, 1979).

The continuous fermentation process was as follows: a strain of *Lactobacillus bulgaricus* was used; the growth medium was constituted with whey or deproteinized whey, to which corn steep liquor was added as a source of growth factor; the pH was maintained at 5·5 by the addition of ammonia; the temperature was maintained at 43°C. Sterilization of the medium was not necessary in view of the pH, temperature and anaerobic conditions used, and the high concentration of undissociated lactic acid. The fermentation lasted from 14 to 24 hours with a lactose–lactic acid transformation yield of 95% for lactose contents below 70 g/ℓ. After concentration to 70% dry matter, the final product contained 55% protein (N × 6·25): 76% of this was ammonium lactate, 17% was whey protein and 7% was bacterial cell protein. This product was termed 'fermented ammoniated condensed whey' (FACW).

The continuous fermentation process has been studied by Reddy, Henderson and Erdman (1976). Two fermenters in series were used at pH 5·5 with a retention time of 31 h; the transformation yield was 98%.

In order to increase the productivity, Coulman, Stieber and Gerhardt (1977) studied a continuous culture process with a dialysis stage. Under these conditions the retention time was reduced by 19 h with a transformation yield of 97%.

Comparison of the three processes showed that the rate of transformation of lactose (mg/ml/h) fell from 4·4 in the batch process to 1·1 in the continuous process. This rate increased to 12·1 in the process with dialysis. It is also noteworthy that this third process used a lactose input concentration of 230 g/ℓ compared with 70 g/ℓ for the batch process and 50 g/ℓ for the continuous culture process. The production of FACW appears to be of great potential interest: Gerhardt and Reddy (1978) have pointed out that, with appropriate adaptation, this could be an excellent process for the treatment of aqueous effluents from many food industries.

PRODUCTION OF BAKER'S YEAST

The production of baker's yeast from cheese whey with its lactose already transformed into lactic acid has been considered (Moebus and Kiesbye, 1975). *Lactobacillus bulgaricus* could transform lactose completely into lactic acid, which would imply the use of large quantities of a neutralization agent. In addition, Dion, Goulet and Lachance (1978) have shown that cell growth is strongly inhibited by high concentrations of lactic acid (above 10 g/ℓ). In order to reduce this inhibition problem, Champagne, Lachance and Goulet (1980) have proposed the use of a strain of *Streptococcus thermophilus*. This strain transforms only part of the lactose content into lactic acid and excretes galactose into the medium. The production of baker's yeast is based partly on lactic acid and partly on galactose as substrates. Under the operational conditions described by Champagne, Lachance and Goulet (1980) at pH 5·5, 30°C and 0·3 vol/vol/min, the dry-matter yield for lactose was 31%.

In 1977, the world production of baker's yeast was 177 000 t. The use of cheese whey to produce such a quantity would involve about 10% of the total world whey production.

PRODUCTION OF ETHANOL

The alcoholic fermentation of lactose was demonstrated in 1947 by Rogosa, Brown and Whittier. However, only a few species of yeast could ferment lactose. Several selection studies of strains capable of fermenting lactose directly have been undertaken (Gawel and Kosikowski 1978; Laham-Guillaume, Moulin and Galzy, 1979; Demott, Draughon and Herald, 1981; Friend, Cunningham and Shahani, 1982; Giec and Kosikowski, 1982; Izaguirre and Castillo, 1982). The strains selected by different authors belong to the species *Kluyveromyces fragilis* or *Candida pseudotropicalis*. In general, the fermentation of lactose up to a concentration of 200 g/ℓ could be performed with over 90% of the theoretical yield. Deproteinized whey can be fermented without any additive, and no variation in pH has been observed during the fermentation process. The fermentation period varied from 4 h for wheys containing 40 g/ℓ lactose to 20 h for wheys containing 200 g/ℓ lactose with recycling of yeast cells. In view of the inhibition mechanisms of alcoholic fermentation in the presence of lactose (Moulin, Boze and Galzy, 1980), the optimal lactose concentration was about 150 g/ℓ; this sugar concentration would yield 8% (v/v) ethanol.

Deproteinized cheese whey could thus be fermented into alcohol under favourable conditions. In 1979, the US Department of Energy in its report on the alcohol fuels policy, indicated that cheese whey is one of the cheapest feedstocks per gallon of ethanol. Other studies published recently (Reesen and Strube, 1978; Moulin and Galzy, 1981; Barry, 1982; Singh *et al.*, 1983) have shown the importance of prior recovery of proteins from cheese whey. These authors also demonstrated that ethanol production from cheese whey is competitive against chemical synthetic processes; in addition, there is a 30–40% net energy gain. At present, ethanol is being produced from deproteinized cheese whey in Ireland (Lyons and Cunningham, 1980; Barry, 1982), and in the USA (Walker, 1982).

Different projects are under investigation in the USA (Chen and Zall, 1982; Singh *et al.*, 1983), in New Zealand (Barry, 1982), and in France.

The production schemes given by Barry (1982) and by Reesen and Strube (1978) showed a continuous fermentation process in two stages, with a 12 h retention time. Yeast cells were partly recycled and some new cells were added. In the process described by Reesen and Strube (1978), the effluent from the distillation process was treated by anaerobic fermentation to produce methane. Following fermentation, the chemical oxygen demand of cheese whey was reduced by 90%: however it remained at a high level (5000–7000 mg/ℓ); further treatment of the effluent was thus required.

It is noteworthy that the development of lactose hydrolysis processes permitted the use of traditional distiller's strains (Kosikowski and Wzozeck, 1977; O'Leary *et al.* 1977a,b). However, the diauxy feature which appeared for glucose and galactose required the selection of derepressed mutants (Baily, Benitez and Woodward, 1982).

PRODUCTION OF GALACTOSE

The production of galactose by chemical means was studied by Clark (1921) and by the Préval Company (Société Préval) (1971). Clark used a chemical hydrolysis of lactose followed by a fractionation extraction of galactose with ethanol. The Préval process recommended the hydrolysis of lactose either by chemical or enzymatic means; glucose could then be eliminated by growing a yeast strain such as *Saccharomyces rosei*. Following fermentation and separation of yeast cells, the medium contained only galactose which could be extracted with ethanol.

The excretion of galactose was observed by Cooper *et al.* (1978) in an *Arthrobacter globiformis* culture growing on a medium containing lactose as carbon source. Similarly, Champagne, Lachance and Goulet (1980) showed that lactose was converted by *Lactobacillus thermophilus* partly into lactic acid and partly into galactose. Furthermore, yoghurt and kefir-type products resulting from lactose fermentation of milk always contain some galactose.

The production of galactose from whey permeate by biological means was proposed by Galzy and Moulin (1976) and Moulin, Varchon and Galzy (1977). *Kluyveromyces fragilis* and *Kluyveromyces lactis* yeast strains containing β-galactosidase are able to metabolize galactose. It should be possible to select mutants which have lost the ability to metabolize galactose while retaining the ability to hydrolyse lactose into glucose and galactose. Such a mutant could thus hydrolyse lactose, grow on the resulting glucose and secrete galactose. The genetics of lactose metabolism regulation in *Kluyveromyces lactis* has been studied by Dickson and Sheetz (1981), Sheetz and Dixon (1980), Carré (1978) and Fuentes (1981). The results showed that such mutants could be selected following multiple mutations. These mutant strains would have lost either their galactose permease galactokinase and epimerase, or their galactose permease galactokinase and transferase (Moulin *et al.*, unpublished results). These mutants could absorb lactose using a lactose permease, hydrolyse it with a β-galactosidase, and metabolize the resulting glucose with a growth rate of 0·3/h and

a 0·45 growth yield (g dry weight per g glucose) from available glucose, with secretion of galactose into the medium. This fermentation was performed at pH 3·2, 32°C and the aeration was set at 1·5 vol/vol/min. The medium was supplemented with a nitrogen source and trace elements (Zn, Mn, Cu, Fe). The production of galactose from deproteinized whey containing up to 60 g/ℓ lactose reached 98% of the theoretical yield. The productivity obtained was 1·5 g/ℓ/h. The solution obtained following fermentation and separation of yeast cells contained only galactose as carbon substrate. Galactose could be extracted as in the processes described above for ethanol.

LACTOSE AND ITS DERIVATIVES

The manufacture of lactose from whey or from ultrafiltration permeates is a well-known process (Krevela, 1969; Nickerson, 1970, 1979). However, the amounts of lactose produced world-wide would require the use of only 5% of the whey available (Coton, 1980). The development of lactose chemistry would help to increase the amount of whey which could be utilized for lactose production. Nickerson (1979) in a review on lactose chemistry showed how this compound could be used for the absorption of flavours or as a complexing agent for metal ions. In addition, lactulose, lactitol and lactobionic acid are three important compounds derived from lactose.

Lactulose ((4-*O*-*β*-1) D-galacto-pyranosyl-D-fructose) is mainly used in pharmacology. This compound can be obtained by chemical means at 35°C under alkaline conditions (Hicks and Parrish, 1980), or by using an ion exchange resin (Demainay and Baron, 1978); these processes have relatively low yields. The production of lactulose from lactose with the enzyme *β*-galactosidase (EC 3.2.1.21) has been studied by Vaheri and Kauppinen (1978). The rates of galactosyl transfer and the biochemical nature of the products obtained vary according to the origin of the *β*-galactosidase enzyme. The *β*-galactosidase from *Kluyveromyces fragilis* could transform only 8% of lactose into lactulose, and other compounds (galactosyl-galactose and galactosyl-glucose) also were produced.

Lactitol can be produced by hydrogenation of lactose with nickel as catalyst (Van Velthuijsen, 1979). Hayashibara (1976) has demonstrated the possible use of lactitol as a sorbitol substitute. According to Van Velthuijsen (1979), lactitol has interesting possibilities in low-calorie diet foods. It is also noteworthy that the esterification of lactitol into lactitol palmitate would yield a compound with interesting emulsifying properties as an additive in foods or detergents (Zadow, 1979). Lactobionic acid, produced by enzymes, could be used as a food acid (Zadow, 1979).

The production of 'lactosylurea' is being much studied for ruminant feeding purposes. This compound is relatively slowly hydrolysed by the rumen microorganisms: this property would allow the increased use of urea as a non-protein nitrogen source in ruminant feeding without running the risk of reaching the toxicity level of ammonia (as when pure urea was included in the ration). This compound is obtained by reaction between urea and lactose.

The conversion of lactose into different products has been attempted mainly

by chemical methods: enzymatic and biological techniques now offer new possibilities to be explored.

HYDROLYSIS OF LACTOSE

The partial or total hydrolysis of lactose is interesting from the viewpoint both of nutrition and of food technology. In addition, bearing in mind the development of ultrafiltration techniques, lactose hydrolysis would be an excellent way of furthering the use of permeates. According to Alam (1982), lactose intolerance is very widespread, affecting 70% of the non-white population of the USA and 95% of the populations of Asian and African countries. Harries (1982) puts the prevalence of hypolactasia at 50–90% in most non-Caucasian races and 2–30% in Caucasians. The limiting factor for using lactose as feed also seemed to depend on its hydrolysis by the animals following weaning (Février and Aumaitre, 1981). The hydrolysis of lactose would help to overcome the enzyme deficiency problem in some species.

From the technological point of view, the hydrolysis of lactose would improve two parameters important for the food industry, namely the sweetening power and the solubility of the sugars: the solubility of a glucose–galactose mixture is 60% whereas lactose alone would have a maximum solubility of only 22%; the solubility of sucrose is 68%. Similarly, the sweetening power of the glucose–galactose mixture is 70, whereas it is only 15 for lactose; again, sucrose is used as reference (sweetening power 100). In addition, hydrolysis of the lactose in the permeate would yield a mixture containing glucose and galactose, which can be metabolized by a greater number of micro-organisms.

Hydrolysis process

The hydrolysis of lactose can be performed either by chemical or by enzymatic means.

Hydrolysis of lactose with catalytic resin. Chemical hydrolysis is achieved by passing the substrate through a cationic resin, in the acidified state, at 90–100°C. Previous demineralization of whey (over 95%) would be necessary (Le Henaff, 1978; Coton, 1980). This process is still at the pilot plant stage and not all the process conditions have as yet been worked out. Furthermore, lactose solutions hydrolysed by this process contain (together with residual lactose, glucose and galactose) small amounts of other compounds such as lactulose, trisaccharides and high-molecular-weight sugars (Coton, 1980).

Several such processes have been put forward by different companies including Applexion Company (France), Portal Water Treatment Ltd (UK), Permutit Co. (UK), Technichem (USA) and Rohm and Hass (USA).

Enzymatic hydrolysis. The β-galactosidase enzyme has been isolated from animals, plants and micro-organisms, but only the latter have been used in the industrial production of β-galactosidase. Bacteria, yeast and fungi are all good sources of β-galactosidase: however there are important differences between the

β-galactosidases from different sources. Their main features have been reviewed by Shukla (1975), Greenberg and Mahoney (1981) and Richmond, Gray and Stine (1981). In general, yeast β-galactosidases are characterized by a neutral pH and are less stable than those isolated from fungi. There are many published studies on bacterial β-galactosidases: however, the possibility of food poisoning due to coliforms precludes the use of bacterial β-galactosidase in food products and food processes. Only β-galactosidases isolated from yeast (*Kluyveromyces fragilis*, or *Kluyveromyces lactis*) or from fungi (*Aspergillus niger*) are used.

The hydrolysis can be carried out with soluble enzymes (Nipjels, 1978; Nipjels and Rheinlander, 1982; Giacin *et al.*, 1974): in this case the enzyme preparation is lost with each production batch. This process has often been used for the lactose hydrolysis of milk. Several such enzyme preparations have been developed in Italy, in Holland, and in the USA (Dahlquist *et al.*, 1977).

In addition, the hydrolysis of milk lactose before cheese manufacture could be of interest in the production of yoghurt, soft curd cheese and matured cheese (Thompson and Gyuriesek, 1974; Gyuriesek and Thompson, 1976; O'Leary and Woychik, 1976; Ramet, 1978), with important advantages to be gained either at the lactic fermentation stage or at the maturing stage.

Application of the hydrolysis process to the treatment of permeates would involve the recovery of soluble enzymes after treatment, because of the costs involved; such an attempt has been described by Brulé (1977).

The immobilization of β-galactosidase for lactose hydrolysis has been the subject of many papers. Richmond, Gray and Stine (1981) have reported that over 50 immobilization systems have been proposed: among these systems, immobilization processes by inclusion (Snam Progetti process) and by covalent bonding (Corning process) have yielded interesting results at the pre-industrial pilot plant stage (*see* Chapter 5 of this volume).

In the inclusion process, *Kluyveromyces fragilis* β-galactosidase was immobilized in triacetate cellulose fibres, and a reactor filled with these fibres was used for the pilot treatment of batches of 10 000 litres of milk: 70–80% of lactose was hydrolysed in 20 hours using 450 g fibres. Marconi *et al.* (1979) have extended this process to the treatment of whey.

The covalent bonding immobilization process has been described by Coton (1980) and by Dohan and Baret (1980). The β-galactosidase used was isolated from *Aspergillus niger*. The enzyme preparation was immobilized on a porous carrier using the glutaraldehyde technique described by Havewala and Weetall (1972). The immobilized lactase showed 500 activity (international) units per gram of carrier at 50°C and pH 3·2–4·3.

A preliminary pre-industrial pilot plant has been tested in England by the Milk Marketing Board: the flow diagram of this pilot plant has been given by Dohan and Baret (1980), and by Coton (1980). Cheese whey was pasteurized, ultrafiltered, demineralized and then hydrolysed by the reactor in a continuous system. The pilot plant achieved 80% hydrolysis at a flow rate of 360 litres/hour, and ran for six months, at 16 h/day and 5 days/week. After concentration, the resulting syrup contained 12% lactose, 22% glucose and 24% galactose (Dohan and Baret, 1980).

A second pilot plant which could treat 500 ℓ/hour was tested in France by

the Union Laitière Normande according to the same process scheme. The results obtained for treating whey from casein manufacture confirmed the potential of the immobilization technology of β-galactosidase by covalent bonds (Dohan and Baret, 1980).

Main applications

Partially hydrolysed lactose syrups have been tested in many foods. They avoid the lactose intolerance problem, and the hydrolysis also increases the solubility and sweetness. In most cases, the best results were obtained with hydrolysed permeates which had already been partly or totally demineralized. De la Guérivière (1978), Lombrez (1978), Coton (1980), Salmon (1981) and Dicker (1982) have listed the main foods in which sucrose or corn syrup could be replaced successfully by hydrolysed lactose syrup: these include toffees, fudge, pectin, creams, jam and cakes. Development of these formulations would soon lead to a marked increase in the use of this technology for the treatment and utilization of permeates.

The availability of deproteinized whey containing hydrolysed lactose would also lead to new and greater uses of these products in fermentation processes. A limited number of micro-organisms are capable of hydrolysing lactose, whereas all micro-organisms are able to use glucose. However, the hydrolysate in fact contains a mixture of glucose, galactose and some residual lactose.

Permeates containing hydrolysed lactose permit the use of many strains for the production of food yeasts. Arnaud *et al.* (1978) have described a two-stage process. In an initial fermentation tower, glucose was used by a very rapidly growing yeast such as *Candida utilis*; *Kluyveromyces fragilis* could also be used for this purpose. These yeast cells were recovered by centrifugation and the medium was pumped into the second fermentation tower where its galactose and residual lactose could be used by flora identical to that described in the Bel process. The general operating conditions were a temperature of 35–38°C, pH 3·2, and aeration 1·5 vol/vol/min. Such a system was of interest in that the first tower could be fed with undiluted permeates (50g/ℓ total sugars).

Bakers' yeast may also be produced from permeates containing hydrolysed lactose, according to Arnaud *et al.* (1978). Bakers' yeast would grow on glucose then on galactose with a diauxy effect; it would thus be preferable to use a batch process. A continuous fermentation process would require an initial fermenter for the utilization of glucose, then a secondary fermenter for galactose and finally a third fermenter for the ethanol formed. Yeast cells also have to be recovered by centrifugation at each fermenter outlet.

Stineman (1978) described a process which preferentially used acid whey containing a mixture of glucose, galactose and lactic acid. The fermentation was performed at 30°C, pH 5·0 with an aeration of 1·5 vol/vol/min.

Various authors (O'Leary *et al.*, 1977 a, b; Baily, Benitez and Woodward, 1982) have considered the prior hydrolysis of lactose for alcoholic fermentation with classic distiller's strains. It would be necessary for this purpose to use derepressed strains which are not subject to the diauxy effect (Baily, Benitez

and Woodward, 1982). These processes have not shown any obvious advantage compared with a direct fermentation process.

Hydrolysed lactose syrup could also be considered as a substitute for 'corn syrup' in beer brewing. According to Coton (1980) experiments have shown that it is possible to replace corn syrup with hydrolysed lactose syrup in beer without noticeable alteration in the taste of the beer.

Conclusions

The problem of cheese whey disposal appeared with the increase in size of cheese manufacture production units. Technological advances have continually encouraged the re-evaluation of suggested solutions of this problem.

The first change appeared at the raw material level. The development of protein recovery processes produced greater quantities of available permeates, with a concomitant reduction in the production of whey concentrate and whey powder for animal feedstuffs or human foods.

The possible uses of permeates are very varied. Some applications would require very large quantities of this product: ethanol production is one such process. Several production units are being developed in the USA (Fermentec, California and Milbrew, Wisconsin), and in Ireland (Carbery Milk). The economic interest of these units depends on their size and the economic situation (Delaney, 1981). If a country decided to convert ethanol into fuel, the volume of permeates available would provide only a small fraction of the necessary raw material.

Another example of permeate use is the production of ammonium lactate (fermented ammoniated condensed whey; FACW) for animal feed. This process is being developed by Color Agricultural Research Co. (Wisconsin) with the approval of the Food and Drug Administration. According to Delaney (1981), the daily production of 600 t of dry matter is expected. This product appears to be a competitor of soybeans for animal feed, which would ensure a very important outlet for permeates.

Some processes, however, call for only small volumes of the permeates available: such processes must lead to very valuable products in order to justify further development, and this is the case for the production of galactose and baker's yeast. Burrows (1979) has reported that fluctuations in the price, the availability and the quality of molasses would endanger the baker's yeast industry: the total replacement of molasses by permeates would require only 5–10% of the whey available. Similarly, the production of derivative compounds such as lactulose or lactobionic acid are excellent ways of using cheese whey: they call for only small volumes of whey and would never have raw material supply problems.

An intermediate situation is that of the production of single-cell proteins from permeates (30 000–35 000 t/year). The yeast cells produced are intended for two very different markets: human food applications are profitable but this market is quantitatively limited; animal feed uses would be of interest only if production costs were sufficiently low for the product to compete with other comparable protein sources. The further development of this product is difficult to predict,

and will be linked with the possibilities of incorporating yeast into foods.

Another important area has opened up in the last few years following advances in the enzymatic hydrolysis of lactose. Hydrolysed whey and milk containing hydrolysed lactose are readily used in the food industry. Hydrolysed lactose syrup is another good example of the further use of permeates (Coton, 1980). The utilization conditions of these products are subject to government regulation in some countries. Luquet (1980) has reported that the use of hydrolysed lactose is unrestricted in Canada, is subject to a simple declaration in the UK and is authorized in Italy; in France, a temporary authorization has been given for some products. Hydrolysis of lactose in milk is necessary for access to some markets such as the Middle East, Mediterranean countries, and various African and Asian countries (Luquet, 1980; Alam, 1982). The novel functional properties of hydrolysed whey also opens up numerous industrial possibilities. The development of hydrolysis processes will depend on the current legislation of different cheese-producing countries.

The utilization of cheese whey and ultrafiltrate in the fermentation industries raises several problems:

1. These substrates with low dry-matter contents are excellent growth media for many micro-organisms. This feature, while constituting the essential advantage of these products, also provides the main problem with regard to their preservation and, subsequently, their transportation.
2. In many countries there is the problem of collecting whey over large geographical areas. Whereas in industrial centres, many large production units have been built often with a daily whey output of 500 000–1 500 000 litres, in other areas (particularly in mountainous regions) there are widely dispersed small units which produce about 5000 litres of whey daily. As on-the-spot treatment is impossible because of the small volumes involved, the cost of transportation of the raw material then becomes prohibitive.
3. In general, milk production is spread over the year, with some exceptions such as of ewe's milk for the production of Roquefort cheese. Thus, cheese whey is available throughout most of the year although sometimes there is a variation in the volume of production, depending on the season.

The factors listed above (dry-matter content, protein content, amounts available and geographical location) greatly influence the price of cheese whey. This dairy product can be either a source of income or a source of pollution. Cheese whey was formerly considered to be a waste product with no commercial value and for small production units it is still an expensive waste. On the other hand, for large production units, cheese whey is just one of the by-products to be obtained from milk and constitutes a source of income. The price of this raw material thus varies greatly depending on whether it is produced by small or large cheese manufacturers. From small plants, whey would be considered as having no value, however its transportation costs will be important. From large plants, the price would be superior to the existing by-products obtained from whey. In 1983, whey powder was sold at US $0·32/kg (September 1983 level); the cost of drying was about $0·27/kg, not counting transportation costs. These

prices vary considerably depending on the market conditions. On this basis there is a return of 0·3 US cents for each litre of whey processed (15 ℓ of whey are needed to produce 1 kg of whey powder). Protein concentrates (35%) obtained by ultrafiltration are being sold at $0·76/kg, at a production cost of $0·63. On this basis, protein concentrates thus gave a return of 0·22 US cents per litre of whey treated (60 ℓ of whey are needed to produce 1 kg of protein concentrate). The resulting ultrafiltrate was considered to be valueless.

In comparison with other carbon substrates, whey lactose can be assimilated only by specific micro-organisms. In addition, some people cannot assimilate this sugar because of their inborn lack of β-galactosidase. It is evident that glucose and levulose are more universal substrates, permitting the multiplication of almost all kinds of organisms. Sucrose is a substrate which is not utilized by many micro-organisms, however more can grow on sucrose than on lactose. Other substrates such as starch or inulin permit the growth of only a very limited number of microbial species. It is noteworthy that cheese whey can be easily treated in order to transform lactose into lactic acid, ethanol, galactose or a mixture of glucose and galactose according to specific requirements. Cheese whey also contains sufficient proteins to constitute an excellent medium for proteolytic strains; in fact, cheese whey or whey ultrafiltrates are complete culture media. Whey and permeates contain all the minerals and trace elements required for the growth of micro-organisms. They also contain water-soluble vitamins (*Table 4*), and thus constitute an excellent basal medium for any type of culture required by the fermentation industry, whereas it appears to be difficult to produce a culture medium with wide applications from starchy materials without a preliminary treatment. For example, malt obtained from germinated barley is an expensive raw material. Beet or cane molasses currently are the most utilized substrates in biotechnology. Their supply is becoming limited and their quality varies according to the sugar extraction technology employed. These products have an advantage over cheese whey in that they are concentrated and easily transported. Other substrates such as chicory and Jerusalem artichoke juices can be used (Guiraud, 1981); these substrates are rich in inulin and proteins and constitute excellent growth media. Although Jerusalem artichoke can be a valuable substrate, depending on previous treatments such as biological hydrolysis of polyfructosan polymers, the production period is limited and storage of the raw material is necessary. Finally, the main advantage of cheese whey is its constant composition arising from its animal origin. Its competitors are plant products with composition varying according to the cultivar, the harvest date, the annual climatic variations, and pest attacks, as well as subsequent treatments and storage conditions. Moreover, cheese whey is an industrial by-product which is immediately available, and only molasses can be considered to be in the same class of raw material which does not require additional investment for production.

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