Chapter II

LITERATURE REVIEW

2.1 Raw Materials

Raw materials must provide carbon, nitrogen, phosphorus, potassium, magnesium, and calcium; traces of sulfur, iron, cobalt, and other minerals; and vitamins and growth factors, in addition to water (Nickerson and Rose, 1956). No attempt is made in this review to cover a comprehensive list of raw materials used in various microbial protein production. Most effort directed to SCP production for human food use are based on carbohydrates subtrates (Barnes, 1976). The carbohydrates are the largest replenishable source of carbon compound which are available for conversion microorganisms into biomass containing protein (Worgan, 1973, 1975). Proteinaceous materials derived from both animal and vegetable sources are also used as raw materials for SCP production. Some of them are protein wastewaters from potato starch plants (Reiser, 1954), and corn steep liquor (Foda et al., 1973). Both of these raw materials are a principal by-products of the starch production from potato and corn respectively. Hydrocarbon is a relatively new and exciting possible substrate in producing food yeasts. The original purpose was to upgrade the fuel by removing long chain paraffins that greatly increased viscosity. But the by product of the reaction was

vitamin and protein rich yeast that were quickly recognized as valuable food and feed (Enebo, 1968; Synder, 1970; Worgan, 1975). Only carbohydrate substrates for the growth of Candida utilis was reviewed.

2.2 Carbohydrates as substrates for the production of microbial protein

The main carbon and energy source used by microorganisms is termed the substrate. Food and feed yeast are produced from a variety of raw materials with widely different processes. The choice of raw materials for yeast manufacture usually depends on their cost and availability. Low-cost raw material suitable for yeast cultivation has so far been restricted principally to sulfite waste liquors, wood hydrolyzates and molasses. Most of the current production is probably still based on these materials (Reed and peppler, 1973; Jarl, 1969). Agricultural wastes from other sources of carbohydrate sometimes used for the growth of yeast. Most of these wastes are supplemented with simple organic nutrients such as nitrogen, phosphorus, and potassium salts according to need. The remaining trace elements, i.e., iron, cobalt, manganese, and copper are usually present as contaminants of the water or of the carbon source in concentration high enough to satisfy microbial growth.

2.2.1 Spent sulfite liquor

Spent sulfite liquor is an inexpensive waste product of the sulfite pulping process in the paper industry. The liquor contains the degradation products of pentosan, lignin and other non-cellulosic components of wood. It contains 2-4% carbohydrate, 1-2% volatile acids, and 4-5% lignin sulphonates (Brown and Fitzpatrick, 1976). The carbon compounds consist mainly of hoxoses, pentoses, and acetic acid but their proportions vary greatly with the type of wood used (Peppler, 1965, 1968). Candida utilis was initially developed for single cell protein production in sulfite liquor because of its ability to use pentoses (Inskeep et al., 1951; Kurth, 1946; Peppler, 1968; Reed and Peppler, 1973). The growth of Torula yeast on pulp waste liquid serves two purposes, in that a useful feed product is made and at the same time a waste-water treatment is effected. This is because the sugar in the pulp waste liquid would cause serious water pollution if the liquid were disposed of by dumping it untreated into natural waters (Wiley, 1954; Brock and Brock, 1973). When the spent sulfite liquor is the starting material, all media prepared were supplemented with various sources of nitrogen, phosphorus, and potassium to provide the required quantity of the nutrient material. This waste pulp is almost totally lacking of these certain nutrient salts. The sulfite waste liquor fermentation requires the addition of liquid ammonia, diammonium phosphate, and potassium choride for the

growth of C. utilis (Inskeep et al., 1951). The phosphate salts such as ammonium phosphate or phosphoric oxide in the form of P_2O_5 are used to supply phosphorus. Potassium always added in the form of potassium choride (Peppler, 1970; Synder, 1970; Prescott and Dunn, 1959). But Semushina and Monakhard (1966) has suggested that the media did not require the addition of potassium choride as their potassium content fully covers the requirement of yeast and the addition of potassium choride had a growth inhibiting effect. Candida utilis does not require the addition of biotin (Reed and Peppler, 1973).

The only large-scale, economically feasible yeast propagation process for vitamin and protein production is the sulfite waste liquor treatment. This process was well described by Inskeep et al. (1951). Since sulfur dioxide, SO_2, is toxic to yeast, stream stripper or a similar process was applied to remove from the liquor (Reed and Peppler, 1973), Neutralization by lime and a settling process further reduced the SO_2 content. The stripper liquor after adjusting the pH with ammonia was supplemented with nutrient materials as described before. This fortified liquor was introduced into yeasty emulsion at the top of the continuous fermenter (Waldhof type) where mixing took place and compress air introduced to give an emulsion of 50% air and 50% liquor (Galoppini, 1974). A 2:1 mixture of sulfuric acid and oleic acid was used as defoamer (Pelsis, 1964). The yeast product harvested by removing liquid at a rate that balanced the

rate of medium in put (Peppler, 1970; Brock and Brock, 1973). Controls of temperature and pH are highly essential. Temperature was controlled at 30°c by cooling coil in the apparatus. The optimum pH was found to be 4.2-4.5. At higher pH the yield was better, but infection might occur. Contamination was reduced at lower pH. The pH was adjusted by ammonium hydroxide or calcium hydroxide. Ammonium hydroxide solution was preferred becaused it reduced the consumption of the added nitrogen (Reed and Peppler, 1973). The Waldhof fermenter system was developed to maintain the aerobic condition needed for efficient sugar removal by the yeast choice (C. utilis). Waldhof type was originated in Germany and has been adopted through out Europe, Western Asia, Japan, and Taiwan (Peppler, 1970).

2.2.2 Wood hydrolyzates

Another source of raw materials for SCP production is acid hydrolyzed wood. The sugar derived from waste wood has been widely advocated as a source of fermentation carbohydrates. The possibility of producing fodder yeast from wood as a means of supplying protein supplements for cattle feed has created unusual interest among cattle producers, especially in forest areas in which such production would provide an apportunity to obtain locally the protein feed that must be shipped into such areas (Harris et al., 1948b). Wood hydrolyzate has been used as a subtrate for yeast growth and industrial alcohol (Synder, 1970).

Candida utilis was produced from wood sugar hydrolyzate (Harris et al., 1948c), still residues (Kurth, 1946) or the combination of the two subtrates (Harris et al., 1948b). The primary hydrolysis products from wood are lignin, wood sugars, and acetic and formic acids. Secondary products, levulinic acid from the hexoses and furfural from the pentoses, are produced by further decomposition of the sugars during hydrolysis of the hemicelluloses and cellulose (Kurth, 1946). The wood sugar mixtures include glucose, galactose, mannose, arabinose and xylose. The complexity of composition varied with the kind of wood (Kurth, 1946; Peppler, 1965; Reed and Peppler, 1973). The sugars produced by the hydrolysis of wood were subjected to alcoholic fermentation to Torula utilis (Harris et al., 1948a), brewer's yeast and S. cereviseae (Kurth, 1946) to alcohol and carbon dioxides. Pentose sugars do not appear to be used in the alcoholic fermentation with Torula utilis but small amounts may be utilized to grow yeast during or after the alcoholic fermentation is completed. The alcohol is removed by distillation. The residual liquor contains xylose and other nonfermentable materials. The production of yeast from such wood sugars had advantages over alcohol production in that practically all the sugars and some of the acidic material are utilized. However, both subtrates (wood sugar and wood sugar stillage) were also used to produce C. utilis. (Harris et al., 1948c; Kurth, 1946). These raw materials contain very few of the requirements for

yeast production except the sugar. Wood sugar solutions contain very little nitrogen or phosphorus and therefore both of these elements have to be added for the growing of yeast. Nitrogen in the form of urea, ammonium sulfate, and ammonium phosphate have been added. The solutions contain small amounts of phosphate, but the phosphate concentration is increased by the addition of the ammonium phosphate. It was customary to add potassium and magnesium salts, these were added without determining the actual need for them (Harris et. al. 1948b). Harris and his assosiates (1948c) found that the addition of magnesium sulfate did not change the yield or rate of growth of yeast. T. utilis was grown without difficulty on the still waste liquors supplemented by 0.1% of urea and 0.05% of KH_2PO_4 (Kurth, 1946). Ammonium sulfate and urea were tried as sources of nitrogen (Kurth, 1946; Peter et. al., 1945). Monobasic potassium phosphate and diammonium hydrogen phosphate at concentrations of 0.05% were used as sources of phosphorus. No variation in yeast yield or rate of growth was observed between the two nitrogen sources or with the concentration of the phosphate salts exceeding 0.05%. Concentration of urea in excess of 0.05% showed no beneficial results in respect to yeast yield or rate of sugar consumption, whereas concentrations of urea in excess of 0.2% retarded cell multiplication (Kurth, 1946). Similar to the sulfite liquor, the wood hydrolyzates most be fortified with K, Mg, phosphate and nitrogen sources (Reed and Peppler, 1973). Experiments were

conducted at the Forest Products Laboratory (Madison, Wis.) to determine the amount of nitrogen, phosphate, potash, magnesium, and sulfite that must be added to the wood-sugar solutions to obtain the maximum growth of yeast. The presence of sulfite during the neutralization of the sugar prior to yeast production improved the yeast yield. The wood species also had the effect upon yeast growth and sugar consumption (Harris et. al.,1948c). The development of satisfactory condition for hydrolysis which would give a product that fermented more readily were conducted at the Forest Products Laboratory (Madison, Wis.) and Oregon Forest Products Laboratory, along with the production of yeast on wood sugar (Harris et. al.,1948a, 1948b, 1948c; Kurth, 1946). The details of the processes were given by Harris and his co-workers (1948a, 1948b, 1948c). For the production of yeast, wood, wood shavings, or saw dust was hydrolyzed with diluted or concentrated acid (HCl or H_2SO_4); after neutralization and clarification, the liquor contained about 3.5 to 4.5% sugars. If the substrate was the still residues the total reducing materials of these liquors calculated as glucose was about 0.9%. The sugar concentration also effects upon yeast yield. So it is usually diluted to the required concentration for good growth. On the basis of sugar being used, a quantity equivalent to 10 to 20% by weight of dry yeast was employed for the propagation (Reed and Peppler, 1973). Assimilation is usually carried out at 31° to 32° C. Good yeast growth occured at initial pH of 4.5

(Peter et. al., 1945). Air diffusion was found to be an important factor in the rate of yeast growth and consumption of sugar. Utilization of sugars was improved by about the same degree as increasing the amount of air had caused. The yield of yeast, however, was higher when oxygen was added than would have resulted by an increase of air (Harris et. al., 1948c). The air consumption per pound of yeast produced was the same magnitude as that required in small equipment for the growth of brewer's and baker's yeasts (Harris et al., 1948b). The amount of air required was also determine by Harris and his coworkers (1948c). It was found that, for yeast production on the still residues, the amount of air was 25 to 50% greater than that required for diluted wood hydrolyzate.

The growth of Torula yeast on wood-sugar solution in conventional equipment is handicapped by inhibiting properties of the solution, and by the tendency to foam excessively, which limits the amount of air that can be introduced (Harris et. al., 1948b). However, detail about these inhibiting substances in wood-sugars was not reported. The partial inhibition of growth in the media could be attributed to the formation of sugar bisulfite adducts which were not utilized by yeast, to the precipitation of lignosulfonic acids on the cell surface (Semushina and Monakhard, 1966). Several investigators have tried to reduce the undesirable foaming properties in wood-sugar solutions (Peter et al., 1945; Harris et al., 1948b, 1948c; Wiley, 1954). The use

of a special fermenter for mechanical control of foam to increase the contact of air with the solution resulted in a completed utilization in a continuous fermenter (Harris et al., 1948c). For yeast production in the conventional type fermenter, it was necessary to dilute the wood hydrolyzate to about 1% reducing sugar in order to obtain satisfactory yeast growth. Continuous feeding of full strength wood-sugar solution to the fermenter has not been practical. At any concentration of sugar, the foaming tendency made it impractical to introduce more than about 0.5 cubic feet of medium per minute until a part of the sugar was utilized (Harris et al., 1948b). It was not necessary to use large amounts of antifoam to keep the foaming under control. Vegifat as a foam breaker on wood-sugar solutions was suggested (Peter et al., 1945). These included oleic acid, corn oil, cotton seed oil, soy bean oil, castor oil, and sulfonated oil (Turkey-red oil). The latter appeared most satisfactory. Batch yeast production in a fermenter with a draft tube and a propeller agitator which caused the liquor to flow down the draft tube made possible control of foaming. Continuous propagation of Torula yeast in a modified Waldhof fermenter appeared to give the most satisfactory operation. Yeast produced in this equipment was cleaner as no antifoam agent was required. More details about the fermenter described above were given by Harris et al. (1948b, 1948c).

The production of wood-sugar to manufacture of food yeast is fairly costly and less economical than that from sulfite waste liquor (Mrak and Phaff, 1960).

2.2.3 Molasses

In addition to sulfite liquor and wood hydrolyzates, another common source of carbohydrates is molasses. Molasses provides the carbohydrates for yeast growth, together with other essential nutrients. Three types of molasses are commonly used: beet molasses, the concentrated syrup left after extraction of sugar from beet juice; cane molasses (blackstrap), the residual syrup after extraction of sugar from cane juice in raw sugar production; and refinery molasses, the concentrated syrup left after refining raw sugar. The choice of molasses type depends upon local availabity, price and chemical composition. Both sugar beet and sugar cane molasses provide the carbon sources (principally sucrose, invert sugar, and raffinose) for assimilation and energy production; considerable amounts of assimilable nitrogen, mainly in the form of organic nitrogen compounds; and many needed minerals, i.e., potassium, magnesium, and trace elements (Nickerson and Rose, 1956; White, 1954; Rose and Harrison, 1971). Cane molasses is usually higher in sugar and vitamin content but lower in nitrogen compounds than beet sugar molasses (Nickerson and Rose, 1956; Sobkowicz, 1976). In some instances carbohydrate-containing raw materials may be used successively for two processes.

Similarly, molasses or wood sugar solutions were used for the production of ethanol with S. cerevisiae and the remaining slops (or vinasse) were further used for the production of feed yeast C. utilis (Reed and Peppler, 1973). The spent wort of a molasses fermentation with S. cereviseae contained unfermented carbohydrates, amino acids, glycerol, and organic acids which were assimilated by C. utilis. (Reed and Peppler, 1973; Leopold and Fencl, 1959). Although both beet and cane molasses contain nitrogenous materials, only a small portions of these are readily assimilated, and in any case the amount is less than that needed for optimum yeast growth. Nitrogen is supplied as ammonium nitrate, ammonium phosphate, or liquid or gaseous ammonia, in addition to the organic nitrogen compounds present in molasses. Mixtures of ammonia and ammonium sulfate are often used in commercial practice, pH control may be readily achieved by varying the ratio of the two. The use of ammonia liquor as nitrogen source reduces foaming during fermentation and decreases the consumption of defoaming agents. Varying the ratios of urea to $(NH_4)_2SO_4$ in the media caused changes in the proportions of each amino acid yield and protein content (Su, et al., 1968). Phosphorus is supplied in the form of calcium superphosphate (Calcium phosphate treated with sulfuric acid), alkali metal phosphate, ammonium phosphate, or phosphoric acid. Phosphoric acid is sometimes preferred for the source of phosphorus because it is able to quickly clarify the molasses solution. This makes it possible

to prepare mashes by a simple continuous process. Phosphoric acid as a nutrient affects favorably physiological state of yeast. The yeast cells are larger and uniform in size. Use of superphosphate is not recommended because it contains inhibitory amounts of fluoride (Frazekas and Sebok, 1959; Brock and Brock, 1973). Other mineral requirements and trace elements for yeast growth are usually satisfied by both beet and cane molasses. However, magnesium may be limiting, and is usually added as magnesium sulfate (White, 1954; Rose, 1961). Alternatively, complex organic sources may be added, e.g. corn steep liquor or yeast extract. The addition of thiamine and calcium-pantothenate raised the yield of yeast and total protein content formed by Torula yeast but the addition of pyridoxine reduced the protein content (Kung-Chin, et al., 1968). It was also found that the presence of each amino acid in the yeast was changed by the addition of vitamins (Su, et al., 1968). Apart from nutritional deficiencies, molasses may sometimes contain substances which inhibit yeast growth. Sulfur dioxide is an important inhibitor in beet molasses which it is removed in practice by heating the molasses prior to clarification. Other inhibitory compounds are hydroxy methylfurfural, potassium imidosulfonate, and various fatty acids. Colloidal substances and secondary metabolic products formed during the propagation also suppress the growth (Burrows, 1970; Almazan et al., 1978). Influence of sugar concentration on the yield of Torula yeast from sugar cane molasses

was studied by Almazan et al. (1972). The yield of T. utilis from molasses decreased from 45-50% to 30-32% when the sugar was increased from 3 to 10%. The concentration of reducing sugars in molasses was therefore, diluted to the range of 2-5% for growing yeast (Matckenko and Krishtul, 1959).

Continuous cultivation is the most economical (Resnis, 1968). The description of the process is largely based on the production of baker's yeast (White, 1954; Pyke, 1958; Nickerson and Rose, 1956; Burrows, 1970). Diluted molasses is clarified after being acidified by sulfuric acid to pH 4.0 and is heated to precipitate calcium sulfate and other suspended materials. The clarified molasses solution supplemented with the inorganic nutrients serves as medium for propagating the yeast in continuous culture.

In summary, molasses needs no potassium, and less phosphorus and nitrogen supplement than sulfite liquor or wood hydrolyzate when they are used for yeast production (Nickerson and Rose, 1956). Nitrogen is supplied in the form of ammonia or ammonium salts, and phosphoric acid or phosphates in a fashion similar to that for baker's yeast (Nickerson and Rose, 1956; Peppler, 1970).

2.2.4 Starch hydrolyzates

Starch hydrolyzates are the liquid wastes from the manufacture of starch from wheat, mize, tapioca, potato, cassava etc.

(Flannery, 1975). These wastes have to be pretreated before used in yeast production because the starch could not directly utilized by C. utilis. They have to be converted to simple sugars by means of hydrolysis or amylolytic enzymes (Kihlberg, 1972; Worgan, 1973; Jarl, 1969). The "Symba" yeast process was developed to produce the microbial protein production from the starch manufacture. The process has lead to economic utilization of these starch wastes. The aim of this process was a conversion of starch into yeast cell mass by growing C. utilis in symbiosis with an amylase-producing yeast species Endomycopsis fibuligera. The enzyme activity of the latter converts the starch into lower saccharides, predominantly glucose, which C. utilis is then able to use for biosynthesis of cell substances. Since the growth rate of E. fibuligera is only moderate, the process yields a product substantially of Candida yeast. More details have been described by Jarl (1969), Worgan (1973, 1975), and Rattakul (1976).

2.2.5 Cellulosic wastes

Cellulose is the main component and is the most abundant organic compound on earth. In wastes such as straw, cereal husks, and other plant residues, they are invariably accompanied by lignin and hemicelluloses. Because of the protective effect of the lignin and the partially crystalline structure of the cellulose, these materials are resistant to rapid breakdown by

microorganisms. When heated with acid, cellulose is converted to glucose and the hemicellulose to pentose sugars. When this hydrolysis is neutralized, it can be used for the growth of food yeast. The alternative to acid hydrolysis of the cellulose is to break it down by means of enzymes (Worgan, 1973, 1975).

2.2.6 Fruit wastes

Most of fruit wastes from food processing is a good source of carbon for C. utilis. The simple sugars in fruit wastes make it more advantageous than the first two substrates (starch hydrolyzates and cellulosic wastes). However, it has a few published reports on the production of C. utilis from fruit wastes. Some of them are apple juice (Goucko and Bujak, 1973), pineapple cannery waste (Reyes and Cassas, 1961), and citrus waste press juice (Nolte et al., 1942). All of the three wastes (2.2.4,2.2.5 and 2.2.6) require the addition of nitrogen, phosphorus, and potassium for yeast to utilize sugar in the media (Reiser, 1954). In using rice husks as substrate, the hydrolyzate was supplemented with KCl, $(NH_4)_2SO_4$, and superphosphate (Savinykh et al., 1967). The addition of nitrogen, as ammonium sulfate, resulted in solid recovery and the sugar removal was substantial increased to 95% in potato starch waste (Sundhagul, 1972). The protein content was increased, and the weight of yeast was also doubled (Worgan, 1975). Organic mutrients such as yeast extract, polypeptone, and meat extract were

beneficial in decreasing COD when used together with $(NH_4)_2SO_4$ (Katsuguki, 1967). Other non-protein nitrogen sources were also employed such as ammonia, urea and ammonium phosphate. Sundhagul (1972) found that yeast growth with added phosphorus in tapioca starch wastewater showed no advantages. But in potato starch manufacture, deficiency in phosphorus nutrition was determined (Reiser, 1954; Oosten, 1975). To satisfy the yeast requirement for phosphorus, a medium with H_3PO_4 and superphosphate were employed to maintain the P_2O_5 at the required level (Stakheev and Posbrebko, 1972). In general, the hydrolyzates vegetable wastes were substantially richer in K than the hydrolyzate of wood (Morozova and Vyrodora, 1974).

It may be concluded that agricultural wastes can serve as excellent substrate for yeast growth but are handicapped in many instances by the cost of collection and pretreatment, and by the unfavorable factor of seasonal supply which does not allow ready amortization of heavy capital investment (Synder, 1970; Wiley, 1954).

2.3 Pineapple and Pineapple Juice

Pineapple or pineapple juice is the most non-citrus juice used in American diet. Recently, the production of pineapple and its juice have expanded in several areas. The chief sourecs of the world canned pieapple products are Hawaii, Phillippines, Taiwan, Thailand, Malaysia, South Africa, Australia, Ryukyas (Okinawa) and Ivory coarst. Other production centers of lesser

importance are Mexico, Puerto Rico, Kenya, and Swaziland (Tressler and Joslyn, 1971). The composition of pineapple fruits and, hence, of the juice depends upon variety, ripeness, season, crop, locality, plant status, weather, fruit portions used and of blemishes. Pineapple juice is a semiperishable product and its quality deteriorates on storage, even at room temperature. Pineapple juice like most other fruit juice, consists chiefly of water, sugar, acids, small quantities of coloring matters, mineral substances, and also containing many vitamins and other known and unknown nutritional factors. The chemical composition of frozen pineapple juice is shown in Table 1 along with a Thai pineapple juice (Smooth Cayennee variety). Sucrose is the predominating sugar, being presented in roughly twice the quantity of the reducing sugars. Fully mature Cayennee pineapple juice contains about two-third sucrose and one-third reducing sugars. The reducing sugars are composed of approximatly equal amount of glucose and fructose. During processing of pineapple juice there is some inversion of the sucrose. This inversion continues during storage, until the sugar is practically all in the reducing form. (Tressler and Joslyn, 1971). From Table 1, it can be seen that pineapple juice contains a fair source of ascorbic acid, thiamin, riboflavin and vitamin B_6. It is also a fair source of potassium but are not for sodium. Pineapple juice is well buffered. The pH of various lots ranges from 3.3 to 3.7. The average pH value of pineapple juice grown in Thailand is

Table 1 Proximate, mineral, and vitamin composition of frozen pineapple juices[1] and Thai pineapple juice[2]

Proximate composition (%)	frozen pineapple juice[1]			(smooth cayennee)[2]
	max.	min.	av.	
solid	15.1	13.4	13.8	
ash	0.39	0.31	0.35	
ether extract	0.04	0.03	0.03	0.03
protein	0.50	0.36	0.41	0.4
crude fiber	0.19	0.08	0.11	0.5
total carbohydrate	14.30	12.6	13.0	14.0
calories per 100 grams	53	47	49	54
mineral, mg per 100 grams				
calcium	12.8	9.0	10.8	22
total iron	0.52	0.24	0.32	0.4
phosphorus	9.6	7.6	8.3	8
magnesium	10.7	4.9	8.9	
potassium	170	135	143	
sodium	1.0	0.6	0.8	
vitamin, mg per 100 grams				
ascorbic acid	16.6	11.0	13.0	17
β - carotene	0.011	0.006	0.009	15 (IU.)
folic acid	0.002	0.001	0.001	
pantothenic acid	0.157	0.094	0.125	

Table 1 Proximate, mineral, and vitamin composition of frozen pineapple juices[1] and Thai pineapple juice[2] (Continued)

Proximate composition (%)	frozen pineapple juice[1]			(smooth cayennee)[2]
	max.	min.	av.	
riboflavin	0.119	0.013	0.126	0.04
thiamin	0.069	0.058	0.066	0.09
vitamin B_6	0.098	0.053	0.074	
niacin	0.29	0.20	0.25	0.2

[1] D.K. Tressler, and M.A. Joslyn, Fruit and Vegetable Juice Processing Technology (Westport, Connecticut: The Avi Publishing Company, Inc., 1971), p. 175.

[2] รายงานการสำรวจสภาพการทำไร่สับปะรดในเขตจังหวัดประจวบคีรีขันธ์ (เอกสารวิชาการที่ 2, กรมส่งเสริมการเกษตร, 2513), หน้า 10–11.

about 3.5 (Anonymous, 1970).

Only one study on the production of yeast on pineapple juice has been published. The press juice of pineapple was first fermented with Aspergillus awomori to yield citric acid (Liu et al., 1957). The maximum yield of citric acid was 17% while the original content was only 0.05%. The waste-water recovered from citric acid fermentation was used for growing yeast (Torula utilis) (Eugenia and Irena, 1968). T. utilis was also produced from pineapple canning industry wastes, cultivated at temperature 30-33°C, pH 3.2-4-8 for 12 hours (Reyes and Cassas, 1961).