

I N T R O D U C T I O N

The increase in the world population with the existing shortage of high quality low-cost foods, makes the recovery of nutrients from the wastes and their utilization as foods or feeds, necessary to narrow the gap between the population and food supplies.

The amount of vegetable wastes seems to be high and could be used to obtain some important products such as, leaf protein concentrate (LPC), single cell protein (SCP) and pectin.

Leaf protein concentrate (LPC) is one of many novel sources of proteins which possesses good nutritional value. However, it is not widely accepted in the human diet. It has been shown to have a favorable balance of essential amino acids (Gerloff et al., 1965; Byers, 1971 and Betschart and Kinsella, 1974)

Single cell protein (SCP) refers to any unicellular source of protein, including yeast, bacteria, fungi, and algae. The value of SCP is in its use as a nutritional supplement where conventional protein sources are not available or uneconomical. The production of SCP can be simply defined as the process of converting raw materials into cellular biomass. The production cost of SCP depends

upon substrate cost, operating expenses, and capital investment for fermentation equipment. Improvements in SCP yields, productivity, and quality can be obtained by optimizing the various stages in the process. Single cell protein is used not only to produce protein but also to reduce the pollution in food plants.

Highly purified pectin became commercially available since about eighty years. Besides the known use of pectin in jams and jellies, new applications developed by using pectin in fruit powders. Pectin can also be used as an effective synergistic antioxydant. An interesting aspects of pectin pharmacology was found to reduce cholestrol levels (Voragen and Pilnik, 1970).

In this investigation, utilization of four vegetable crops by-products (carrots, cauliflower, watermelon and artichoke), were studied.

Aim of investigation:

The present investigation was carried out to study the following:

- 1- Some aspects of the preparation of leaf protein concentrates from haulm and leaves of some vegetables, especially those which are not utilizable to any extent and thus considered as waste, as well as the most abundant plant weeds in Egypt.

REVIEW OF LITERATURE

A. Chemical composition of raw materials:

In general, haulms and leaves of the different plants showed great variation in chemical composition, especially moisture and protein content.

Ahmed (1971) mentioned that the chemical composition of watermelon juice was as follow: total soluble solids 7.27%, reducing sugars 5.72%, non-reducing sugars 0.12%, protein 0.35%, ash 0.24% and acidity 0.12% (as citric acid).

Hassan (1983) reported that dry matter, crude fat, crude protein, carbohydrates, crude fiber and ash contents of artichoke wastes were 24.8, 1.2, 14.3, 48.4, 29.5 and 6.6%, respectively (on dry weight basis).

Higazy (1984) found that the chemical composition of cauliflower was 86.1, 4.3, 0.2, 1.9 and 26% for moisture, protein, fat, ash and fiber, while the chemical composition of carrot was 85.4, 3.9, 0.4, 2.0 and 31.0%, respectively.

Gazal (1989) determined the chemical composition of carrot haulm and potato leaves. He found that the dry matter, crude protein, ash, ether extract, crude fiber and total carbohydrates were (13.14 and 13.61), (23.80 and 28.13), (12.14 and 26.57), (2.92 and 2.21), (21.16

and 22.04) and (61.64 and 43.10%), (on dry weight basis), respectively.

Radwan (1989) found that watermelon fruits contained 52.797% pulp, 45.099% peels and 2.14% seeds. The juice contained, 6.73, 6.5, 5.36 and 0.858%, total solids, total soluble solids, total sugars and ash, respectively.

Bayoumy et al. (1990) found that the chemical composition of pea haulm was as follow: dry matter, crude protein, ash, ether extract, crude fiber and total carbohydrates were 16.67, 22.38, 10.68, 4.14, 25.43 and 62.80% (on dry weight basis), respectively.

B. Protein from plant leaves (Leaf Protein Concentrates):

Protein obtained from vegetative plants represents a potential sources of human food. The acceptance of leaf protein concentrates as human food has been retarded by objections associated with their green colour, grassy flavour and lack of solubility. However, during the past few years there has been a renewed interest in protein preparations intended for human food, (Woodham, 1965 and Singh, 1971). The lysine content of (LPC) which ranged from 6 to 7 g/100 g protein, accounts for its effective use as a supplement for diets based on vegetable proteins which are commonly low in lysine, (Doraiswamy et al., 1969). LPC has been used also as a milk extender for infants and

young children (Waterlow, 1962). Green stated that LPC, besides being a high quality protein source, was also a good resource for poultry egg yolk and skin pigmentation (Kuzmický et al., 1977).

Pirie (1969 a & b) emphasized that green parts of plants containing a protein source that is only utilized to a very small extent for direct human consumption if properly processed. Leaf protein concentrates can, however, be a nutritionally valuable supplements to protein coefficient foods. Waste green crops such as beet tops, pea vines, small etc. can be used in the leaf protein process (Oelshlegel et al., 1969).

1. Juice separation from green leaf materials:

In order fractionation for green crop, to be widely adopted, it must be possible to, inexpensively, expressed plant juice at reasonably high rates. This requires a knowledge of the concepts of cell rupture and juice expression. The nature and requirements of these processes have been investigated by various workers. Jones (1977) had compiled informations on the principles of green crops fractionation and has identified some areas where additional research is needed.

Cell rupture and juice expression should be carried out separately (Koegel et al., 1974). This makes the entire

plant liquid available for flushing out the chloroplasts which tend to be released somewhat later in the rupturing process. The chloroplasts are a major source of protein in the expressed juice.

Schwartzberg et al. (1977) mentioned that roughly 88-95% of the protein containing cells in alfalfa are opened by vigorous maceration but, because of pressed cake blinding, only 65-75% of the mobile protein content (46-53% of the total protein content for alfalfa, 80% of its protein is mobile) that can be recovered by simple, single-stage pressing. However, almost all of the remaining mobile protein in the open cells can be recovered by multistage addition of water and expression following the first pressing. The protein concentration reductions caused by such rewetting and expression can be minimized by carrying out the process in a counter current fashion. Aproximately 20% of the protein in the alfalfa appeared to be immobile and susceptible to recovery only by chemical or enzymatic treatment.

Edwards et al. (1978) reported that grinding substantially increased the yield of leaf protein concentrate (LPC), extracted dry matter, extracted crude protein, crude protein recovery, and pressed cake dry matter content from fresh alfalfa dewatered in a twin-screw press. Average LPC yield of 15.2% (dry basis) can be obtained

from ground alfalfa by using the twin-screw press. Grinding of wet, field-chopped alfalfa can be accomplished without clogging in existing commercial hammer mills of appropriate design. The yield of LPC and other processing results can be predicted from a multiple linear regression equation if the appropriate raw material and processing parameters are known.

Hamza et al. (1983) found that the green juice of berseem and alfalfa was produced by pulping and pressing. Fractionation of the chloroplasts and the chloroplast free juice was performed by direct heat through heated oil. The juice was then separated by centrifugation and the white protein was precipitated by acidification. Amino acids were determined in the green and white protein products. Results revealed that high quality protein products could be obtained from berseem and alfalfa juice, while the press-cake left after juice extraction contained enough protein for animal feed.

2. Factors affecting juice recovery from starting plant materials:

A wide variety of factors influencing juice and protein recovery during juice expressing from macerated alfalfa leaves. They included (1) cell rupture, (2) fragmentation of protein containing cell organelles, particularly chloroplasts, (3) pressing rates during expression, (4)

press cake mass per unit of outflow area, (5) juice viscosity, (6) blinding of the expression outflow medium, (7) organelle and juice entrapment within pores which are blocked during the compaction that accompanies expression, (8) soluble protein coagulation and chloroplast flocculation prior to and during expression. Schwartzberg et al. (1977) indicated that all of the above factors are important.

Hanna and Ogden (1980) reported that the largest differences in protein yield could be attributed to varietal differences for the leaves involved, substantial yield differences which appear to be due to the differences in growth and harvesting conditions. They added that juice protein yield had been empirically correlated with various processing, harvest, and growth-related factors.

3. Protein recovery from juices of green leaves:

3.1. Methods for protein extraction:

During the last three decades, a great deal of research activity had occurred in the area of extraction of leaf protein from various sources. Pirie (1971) published his results on agronomy, nutritional value and extraction methods for leaf protein concentrate and described large-scale extraction and processing methods. The primary purpose of the concentrate was to supplement human foods. Workers at the U.S.A. Department of Agriculture, Western Regional Research Laboratory. Kohler et al. (1968), Spencer

et al. (1970) and Edwards et al. (1975) have developed two processes for preparation of LPC. In the first process, the expressed plant juice was heated to coagulate the protein, thus providing a concentrate suitable for feeding nonruminants. In the second process, the plant juice was separated prior to heat coagulation. As a result, a green concentrate suitable for supplementing poultry rations and a white protein concentrate which could be incorporated into human food were produced.

Several procedures have been recommended to prepare leaf protein concentrate (LPC). Protein in either the green or brown plant, expressed juice that can be coagulated by aging, adjustment of pH to 3-4, application of heat, a combination of heat and acid, or by the use of organic solvents (Chayen et al., 1961; Morrison and Pirie, 1961; Huang et al., 1971 and Lazar et al., 1971). A combination of pulping is generally recommended to extract juice from the leaves. Following the juice separation from the fibrous material, it can be filtered or centrifuged to remove suspended solids. Centrifugation will separate cytoplasmic from chloroplastic protein (Festenstein, 1961 and De Fremery et al., 1973). The juice was opaque brown in colour, after.

Wang and Kinsella (1975) studied yield and protein content of freeze-dried alfalfa leaf protein, which was extracted by different extractants and 71-85% of protein

present in the extract was recovered by acid precipitation. Slightly more protein was precipitated from the water and NaOH compared to the NaCl or Tris extracts. The higher protein yield from alkaline extraction is well recognized (Lu and Kinsella, 1972). Though the more alfalfa leaf protein was extracted with Tris buffer, its protein content was lower than other samples. Therefore, Tris buffer exhibited no advantage over either water or NaCl as extractant under these conditions. The protein yield from NaCl was significantly lower than that obtained with NaOH.

The selection of appropriate plants and pH (Lundborg, 1980) and the use of flocculants (Knuckles et al., 1980a) or calcium salts (Gwiazda and Saio, 1981), combined with very high centrifugal forces (10,000-30,000) have sometimes increased the yield of white protein.

3.2. Methods of precipitation:

Centrifugal forces needed to separate the chloroplastic materials from fresh leafy juice is very high (RCF maximum 100,000 g for 1 hr) and machines capable of producing these conditions on a continuous large-scale basis are not always available. Therefore, centrifugal separation on a large-scale requires some type of pretreatment (Knuckles et al., 1980b). Usually a mild heat pretreatment (40-70°C) is used to agglomerate the green fraction so that it can be easily separated from the soluble protein (Cowlshaw et

al., 1956; Henery and Ford, 1965; Byers, 1967; Subba Rau et al., 1969; Lexander et al., 1970; De Fremery et al., 1973; Bichoff and Kohler, 1974 and Edwards et al., 1975). The temperature necessary to agglomerate the green fraction and the amount of soluble protein which coagulates at these temperatures depend upon the type of plant (Bahr et al., 1977). When alfalfa extracts are heated to 56-65°C, the green material can be separated in large-scale continuous centrifuges but, at these temperatures, 20-40% of the soluble protein is coagulated and separated as part of the green fraction (De Fremery et al., 1973; Edwards et al., 1975). Hill (1965) and Subba Rau and Singh (1970) reported that precipitation of the "cytoplasmic" fraction was maximum at pH between 3.0 and 5.0.

Hood and Brunne (1975) suggested that, the employing of mild heat treatment (60-65°C for 10 minutes), should be applied for removing the chloroplast fraction of alfalfa leaf protein.

Pirie (1975) reported that heating at 70°C is sufficient for complete coagulation, but it is often advisable to heat at 100°C to inactivate the enzymes in the extract. The importance of this inactivation depends on the species of plant extracted and on the distribution of enzymes in it.

Free and Satterlee (1975) reported that production of chloroplastic leaf protein concentrate from alfalfa press fluid using direct centrifugation, with heat (75°C) and acid (pH 4.0) to obtain the cytoplasmic leaf protein concentrate (LPC) fraction.

Bray and Humphries (1978) mentioned that, the yield of "white" protein obtained under optimal conditions of pH, temperature and solvent concentration was relatively low.

Habash (1983) used different methods to prepare LPC from both sugar beet tops and potato haulm. He used heat precipitation method at both 53 and 80°C. Chloroplastic and cytoplasmic protein were isolated by heating to 80°C. Higher yield was obtained by the centrifugation of 10,000 r.p.m. gave an intermediate amount of chloroplastic protein. Precipitation of cytoplasmic protein by acid was markedly influenced by pH 4.0. Minimum solubility occurred at pH 4.0 for sugar beet tops LPC and at pH 3.5 for potato haulm LPC.

Gazal (1989) found that the unfractionated-leaf protein concentrate-heat precipitation (UNF-LPC-HP) prepared from both juices of potato leaves (PotL) and common hyacinth weed whole plant (WHya) exhibited the highest LPC weight or amount [15.23 and 16.63 g/100 g juice (on wet basis)]

or 55.22 and 81.04 g/100 g juice (on dry basis), respectively]. UNF-LPC of PotL and WHya exhibited the highest protein yield (94.59 and 89.44%, respectively) and LPC protein weight obtained as g/100 g of starting materials [2.10 and 1.44 (on wet basis) or 30.38 and 64.75 g/100 g (on dry basis), respectively].

4. Chemical composition of LPC:

4.1. Nitrogen and crude protein:

Byers (1961) extracted protein from the fresh leaves of 60 tropical species, and found that the legumes were the best source of easily extractable and good quality protein.

Subba Rau et al. (1972) found that nitrogen content of LPC varied widely from about 3.8% in carrot to about 9.6% in lucerne.

Romero and Balen (1979) extracted LPC from green peas leaves and the protein content reached 66%.

Sun et al. (1979) mentioned that, the protein content in leaf protein concentrates of vegetables ranged from 26.9 to 73.7%.

Cheeke et al. (1980) prepared leaf protein concentrate from several tropical legumes and found that the crude protein ranged from 28.2 to 59.3%.

Table (1): The protein percentage in leaf protein concentrates (LPC) of various plant leaves.

Type of leaves	Origin	Protein % in LPC	References
Peas peel <u>Pisium sativum</u>	Egypt	28.87-57.96	Radwan (1989)
Potato leaves <u>Solanum tuberosum</u>	Egypt	39.26-66 53	Gazal (1989)
Sweet potato <u>Ipomoea batatas</u>	U.S.A.	35-45	Walter <u>et al.</u> (1978)
Water hyacinth <u>Eichhornia crassipes</u>	Egypt	30.67-53.12	Gazal (1989)
Cabbage <u>Brassica oleracea</u>	Korea	29-32	Choe <u>et al.</u> (1970)
Cactaceae <u>Pereskia aculeate</u>	Barzil	25.10-51.10	Dayrell and Vieira (1977)
Cassava <u>Manibot utilisima</u>	Nurib	38-41	Tupynamba and Vieria (1979)
Alfalfa <u>Medicago sativa</u>	U.S.A.	51-67	Wang and Kinsella (1975)
Alfalfa "	Italy	40.3-48.3	Fiorentini <u>et al.</u> (1980)
Alfalfa "	U.S.A.	about 50	Knuckles <u>et al.</u> (1980b)

Gebre and Sumner (1983) found that total curde protein from pea flour and soybeans were 13.6% and 39.8%, respectively, with protein yields of 43.0 and 55.5%.

4.2. Lipids and fatty acids:

Different values of fat content are mentioned in the literature for the different LPC sources:

Lima et al. (1965) reported that the yield of fatty acids was ranging from 2.5-8.4% in different LPC samples (turnip and rye grass). They added that unsaturated fatty acids formed 53.4-78.7% of total fatty acids in leaf protein lipids.

Lima et al. (1965) and Betschart (1977) indicated that LPC lipids were quite susceptible to oxidation during storage because 53-77% of the fatty acids were polyunsaturated. Leaf lipids are rich in doubly and trebly unsaturated fatty acids. The dominant acids are palmitic, palmitoleic, stearic, oleic, linoleic and linolenic (Pirie, 1978).

Gazal (1989) found that the ether extract contents, the UNF-LPC of potato leaves and water haycynth contained 8.80 and 10.59% lipids on dry basis, respectively. Potato leaves CHL-LPC preparations contained from 5.54 to 15.7% lipids, on dry basis, depending on method of preparation. CYT-LPC from potato leaves and water hycynth preparations

showed lower lipids than CHL-LPC where they had a range of 1.20-3.27 and 2.46-8.01% lipids, respectively.

4.3. Carbohydrates:

The amount of carbohydrates in leaf protein depends on the species and weather at the time of harvesting. Pienazek et al. (1975) suggested that, conjugation with carbohydrates may be one of the factors making methionine unavailable.

Mohan and Srivastava (1981) reported that carbohydrates ranged from 37.37 to 49.89% in the LPC extracted from different species of trees. They also mentioned that, carbohydrates content was 28.75% in LPC from sugar beet leaves.

4.4. Ash content:

Huang et al. (1971) reported that materials prepared from different species of plants precipitated by direct heat (75°C, 5 minutes) and steam injection contained 6.0% ash. Pirie (1971) mentioned that ash content in LPC reached 3.8%.

Spencer et al. (1971) mentioned that determination of ash in LPC is important because of their significance with respect to nutritional value and their possible impact on functional effect of these proteins.

4.6. Amino acid composition:

Many investigators determined the amino acids of LPC and showed that, it has an adequate amount of all essential amino acids especially lysine, however, the limiting amino acid was methionine (Henery and Ford, 1965; Wilson and Tilley, 1965; Hancrakowski, 1977 and Pirie, 1978) Lysine because of many reactions which render it unavailable, and methionine, which is often limiting, are especially important in this respect.

Noguchi et al. (1978) and Rao et al. (1967) showed that all unfractionated leaf proteins were rich in lysine, hence it amounted more than one and half than that of the FAO/WHO reference protein (1973).

Methionine is the limiting essential amino acid in all LPC and its addition improves diet containing unfractionated protein (Shurpalekar et al., 1969). Adding lysine or isoleucine to the diet has no effect and when either is combined with methionine the total response is no more than with methionine alone.

Miller et al. (1975) and Woodham and Dawson (1968) stated that there are many possible reasons for the unavailability of the sulphur containing amino acids in LPC. Heat processing is known to depress the availability of methionine in certain food-stuffs. Decreased availability

is more conventionally associated with Millard reaction between the α -amino group of lysine and aldehyde groups, which occurs on heating many LPC rich in reducing sugars (Pirie, 1978).

Usha et al. (1976) analyzed leaf protein concentrate (LPC) prepared from cauliflower leaves for crude protein, calcium, phosphorus, iron, B-carotene and some of the amino acids. They found that lysine and tryptophane were present in nutritionally adequate amounts, but cystine and methionine were not. Cauliflower LPC was evaluated for its nutritional quality with of growth rate, protein efficiency ratio (PER), liver nitrogen, and plasma protein content by feeding rats. The growth rate of rats and PER values of leaf protein supplemented diets were better than that of the wheat flour fed group. Hence, cauliflower LPC might be used for improving nutritionally poor diets.

Moharram et al. (1981) estimated the amino acids pattern and the chemical composition of the protein extracted from artichoke bracts. The results showed that the artichoke bracts contained 4.36% crude protein, which contained higher level of phenylalanine, tyrosine, histidine, alanine, glycine and serine as compared with the amino acids of casein. The essential amino acids of this protein were similar to those reported in the FAO

provisional pattern. They added that the addition of 1-3% of this protein isolate to biscuits improved its organoleptic properties.

Gebre and Sumner (1983) showed that the amino acid composition of the pea crud is quite close to that of soybean. They also found that addition of gluten improved the sulphur amino acid profile but reduced the lysine content. Flavour of pea and soybean curds was similar, but texture and colour of pea curd was scored lower. Gluten modified the texture and the colour of the curds.

Nasi (1983) found that the LPC extracted from grass, clover, and pea in pilot had fairly high content of lysine, and methionine.

Radwan (1989) found that the chemical composition of unfractionated and chloroplastic proteins extracted by either heat at 53°C or 10°C were well balanced and contained high level of most essential amino acids. They were lower in sulphur containing amino acids, methionine and cystine.

Bayoumy et al. (1990) found that the total amino acid content of CHL-LPC and CYT-LPC for pea haulm were ranged from 37.80 to 40.35 and 39.65 to 44.65 g/100 g protein, respectively. The acid precipitation method showed the

higher total EAA in both LPC types. Sulfer amino acid was the limiting amino acid in LPC samples.

Table (2): Amino acid composition of different whole leaf protein concentrates.

Amino acids	Alfalfa By Byers (1971)	Cabbage By Byers (1971)	Cassava By Otoul (1974)	Cauliflower By Shukla (1978)	Radish By Shukla (1978)	Tobacco By Kung and Tso (1978)
<u>Essential:</u>						
Lysine	6.85	7.1	6.7	10.41	6.0	6.0
Phenylalanine	6.14	6.2	5.9	1.6	-	-
Methionine	1.97	1.9	1.6	5.1	-	-
Threonine	5.12	5.2	4.8	9.8	5.2	5.2
Leucine	9.57	9.3	9.9	10.7	-	-
Isoleucine	5.29	4.6	5.2	-	-	-
Valine	6.37	6.1	6.6	2.7	8.0	8.0
Tryptophan	-	-	-	1.9	-	-
<u>Non-essential:</u>						
Arginine	6.43	6.4	6.7	6.7	-	-
Histidine	2.33	2.4	2.4	6.1	-	-
Tyrosine	4.46	4.7	4.0	-	-	-
Cystine	0.63	2.1	-	-	-	-
Aspartic	10.56	10.0	10.3	-	-	-
Serine	4.69	4.5	4.2	-	-	-
Glutamic	11.38	11.9	13.0	-	-	-
Proline	4.81	4.7	5.9	-	-	-
Glycine	5.65	5.4	5.8	-	-	-
Alanine	6.29	6.1	5.7	-	-	-

Values are reported as grams amino acid per 16 gram nitrogen

of lignocellulosic materials to enzymatic action. An ideal pretreatment would accomplish reduction of lignin content concomitant with reduction in crystallinity and an increase in surface area.

Many different pretreatments have been attempted and the literature on this subject is voluminous. The different pretreatments can be classified into physical, chemical and biological methods depending on their mode of action.

1.1. Acid treatment:

Production of SCP was carried out from maize cobs and stalks by (El-Nawawy et al., 1965 a & b; 1966), from bagasse pith, by (El-Nawawy, 1970) and from rice hulls by (El-Nawawy et al., 1974). Each of these materials was hydrolyzed by dilute acids, then the hydrolyzates were supplemented by some nitrogen and phosphorus salts to be suitable for growth of some pentose utilizing yeasts. Heating the powdered maize cobs with 0.1 N H_2SO_4 (solid : liquid ratio, 1 : 10) at 134°C for 60 minutes resulted in maximum conversion of hemicellulose to sugars, mainly pentoses, and amounted to 33% of the cobs dry weight, besides the degradation of some pentoses (2.2%) to furfural (El-Nawawy et al., 1966). Similar conclusions were obtained with bagasse pith, where 25-40 of the pith were converted to sugars, after heating with 0.2 N H_2SO_4 at 127°C for

60 minutes. Higher acid concentrations (2.0 N) resulted in higher conversion of pentosan and hexosan to sugars (33.2%) of the pith (El-Nawawy et al., 1970). Rice hulls were subjected to 54 treatments of hydrolysis by HCl or H₂SO₄ at different concentrations for different periods and temperature (El-Nawawy et al., 1974). Among these treatments 15% of the ground hulls were converted to reducing sugars after heating with 0.5 N H₂SO₄ for 15 minutes at 134°C.

Han and Callihan (1974) reported that if acid treatment could improve the digestibility of residual cellulose it would be more economically advantageous than NH₃ treatment because an extra addition of a nitrogen source to the growth medium would no longer be necessary. The acid treatment may be also advantageous by producing soluble carbohydrates and partly hydrolyzed cellulose that are readily usable not only for the cellulytic organisms but also for non cellulolytic organisms including yeast.

Wasef (1984) used sulphuric acid in hydrolysis of ground rice husks into two manners. The first manner included sulphuric acid 0.5 N (solid liquid ratio 1 : 10) and hydrolysis was undertaken at 1.5 and 2.0 atmospheric pressure for 15 minutes, according to Sakai (1951). The second manner was according to Smith (1969) who used

sulphuric acid 1.0 N (solid liquid ratio 5 g : 100 ml) and the mixture was heated on a boiling water bath for 6 hours. He found that the first manner was better than the second one.

Shaker et al. (1985) selected an active strain belonging to the genera of Aspergillus sp. for the production of cellulolytic enzymes and proteins when was grown on, peracetic acid, treated wheat straw. It produced considerable amount of carboxymethyl cellulose.

Nour El-Dein et al. (1986) tested different cellulolytic fungi isolates on maize residue (cobs and stems) before or after acid hydrolysis to obtain SCP. They found that Trichoderma (58) and Penicillium (34) gave the highest productivity being 205.98 and 366.55 mg/100 ml, respectively, using maize cobs and maize stems media. Trichoderma sp. (19) and Fusarium sp. (151) gave the highest productivity (129.46 and 170.13 mg/100 ml) using maize stems and maize cobs hydrolyzate media, respectively.

Ali (1988) used concentrated HCl for inversion of bagasse at boiling temperature for 2.5 hrs. The filtrate obtained from hydrolysis process was neutralized with calcium oxide, in amounts varying according to the concentration of acid used in hydrolysis. The neutralized filtrate was filtrated to separate the calcium salts formed.

The hydrolyzate was provided with ammonium sulphate (0.22 g/g sugar) and dipotassium phosphate (0.07 g/g sugar) to adjust the pH to 4.5 and then filter sterilized before the inoculation with the cellulolytic fungi Aspergillus niger and Asp. terreus.

2. Factors affecting fungal growth and protein production:

Gray and Abo-ElSeoud (1966) found that, urea was a better nitrogen source than either ammonium chloride or ammonium nitrate.

Due to the fact, that any waste product containing 3% or more carbohydrates can be fermented (Robinson, 1952). Several agricultural wastes can serve as raw materials for the protein synthesis of fungi. Geethadevi et al. (1978) isolated and screened 26 cellulolytic microorganisms of different genera such as Aspergillus, Fusarium, Penicillium, Trichoderma and Trametes for the production of single cell protein. The protein content of the products produced by these cultures from alkali-treated rice straw was in the range of 7.11 to 41.09%.

Sitaram et al. (1978) studied the ability of some fungal cultures, 5 strains of Asp. niger, 1 strain of each of Penicillium chrysogenum and Pestalotia sp. to produce microbial protein on 3 different pretreated cellulosic substrates (rice straw, bagasse and groundnut shells).

P. chrysogenum strain st-F-3B was the best organism for this purpose and it produced maximum protein when grown on alkali-treated rice straw (ATS) as substrate. Maximum protein of 85 mg/g substrate was produced after 62 hrs incubation on rotary shaker, when the initial pH was kept in the range of 3.5-6.0 at 1% W/V substrate level.

Abd-Allah (1981) studied the production of SCP by certain fungal strains grown on the upper unused part of artichoke bracts and cabbage leaves. He found that Aspergillus sp. (48), Rizhopus sp. (35) and Mucor mucedo were grown optimally at 25°C, while the optimal incubation temperature for Aspergillus sp. (47), Penicillium sp. (6) as well as Mucor sp. (9) was 30°C. She added that the mycelial yield of most of the fungus strains increased slightly by increasing the pH from 4.0 to 5.0. However, increasing the pH from 5.0 to 6.0 seemed to had no effect. On the other hand, some strains were not affected by the pH medium. The same author also observed that increasing ammonium sulphate concentration from 0.6 to 1.0% lead to slightly decrease in the mycelial yield of most investigated fungal strains. Asp. sp. (48) was the only investigated strain which gave a positive relation between mycelial yield and the increment of ammonium sulphate concentration from 0.6 to 1.0%. The mycelial increment rate was 14.6%. Increasing the ammonium sulphate concentration to 1.5% had a very little effect on the mycelial yield of Asp.

sp. (48), Asp. sp. (47), Pen. sp. (6) and Rhi. sp. (35), while it had a negative relation for Rhi. cohnii (222), Mucor mucedo (224) and Mucor sp. (9). Concerning the protein percentage of the investigated fungal strains, increasing the ammonium sulphate concentration from 0.6 to 1.0% which increased the protein percentage by a rate of 8-25%. Total protein yield was increased by a rate of 27.0% in the case of Asp. sp., while the increment rate in other fungal strains was between 1 and 23%. However, more ammonium sulphate concentration (1.5) showed also a negative correlation in case of Asp. sp. (48), Asp. sp. (47), Rhi. sp. (35), while the other fungal strains showed no response. The cultivation of different strains in the medium containing urea, resulted in a good yield of both mycelium and protein ranging from 4.8 to 16.1 and from 1.7 to 5.2 g/l., respectively. The highest mycelial and protein yields were observed in case of Asp. sp. (47), followed by Pen. sp. (6) and Asp. sp. (48). Their yields were 16.1, 13.2 and 12.5 g/l. for mycelial yields, as well as 5.15, 3.96 and 2.63 g/l. for protein yields, respectively. In spite of the great differences between Asp. sp. (48) and Rhi. sp. (35) in their mycelial yield and nitrogen content, the yield of protein was nearly the same. lower protein percentage in Asp. sp. (48) was compensated by its higher yield in mycelium. These results support the findings of El-Shimi (1977) who found that increasing the mycelial yields was accompanied by decreasing

the percentage of crude protein (the media contained about 2.5% sugars).

Miller and Srinivasan (1983) studied the cellulose fermentation with a thermotolerant strain of Asp. terreus. Batch cultivation of Asp. terreus using purified or complex cellulose showed that 80-88% protein content in the biomass ranged from 23 to 38%. Semicontinuous cultivation studies, in which 90% of the biomass was withdraw at the end of the growth cycle, indicated that 84% of added cellulose was utilized with the biomass containing 32% crude protein. Continuous cultivation of Asp. terreus showed that 78-84% cellulose consumption occurred at growth temperatures ranging from 35 to 45°C. Maximum specific growth rates (0.14 per hr.) occurred at 40 and 45°C with a minimum doubling time of 4.9 hr.

Abouziied and Mostafa (1985) reported that three strains of fungi, Asp. niger, As. foetidus and Asp. awamori, beside one strain of yeast, Saccharmycopsis fibuligera, were tested for direct conversion of potato processing waste (PPW) into single cell protein. It was found that Asp. niger is the promissing microorganism for that purpose. The anylolytic yeast tested Saccharomycopsis fibuligera was found to be a practical strain for utilizing (PPW) and producing adequate amounts of cell biomass and protein. The gelatinization of strach and culture shaking has

considerable influences on cell biomass and protein production. Basal (PPW) medium containing peptone, 0.1%, yeast extract 0.2%, malt extract 0.1%, potassium chloride 0.5%, ammonium phosphate 0.2%, magnesium chloride 0.1%, calcium carbonate 0.2% and ferrous sulphate 0.01% was found to be a better culture for production of SCP than Czapck's medium and 2% (PPW) is the optimum concentration for protein production by Asp. niger.

Mourad (1985) studied the protein production using pretreated beet pulp. He found that urea was the most suitable nitrogen source for both Myrothecium verrucaria, and Trichoderma viride at final concentration of 0.14% and 0.07%, respectively.

Solak et al. (1987) investigated the filamentous fungi of genera Aspergillus, Fusarium, Byssochlanyis, Penicillium, Paccilomyces and Trichoderma. They found that the strain Asp. oryzae was the most suitable for the biosynthesis of protein from starch-containing raw-materials. Selection afforded four monocultures characterized by protein increases of 3.1-3.8 g/l. and by protein yield (calculated per utilized starch) ranging from 24.7 to 31.1% and medium utilization fluctuated between 74.0-84.9%.

Ali (1988) observed a decrease in ammonium nitrogen and increase in organic nitrogen during Asp. niger and Asp. terreus propagation on pretreated bagasse, and

consequently crude protein indicated that the tested fungal strains grew with different rates. Apparently, these variations in growth rate of investigated strains reflected the variation in their activities and abilities to decompose the bagasse materials. The variation in growth was more pronounced when treated bagasse samples were used as compared with native bagasse.

Radwan (1989) found that the suitable conditions to produced single cell protein from watermelon juice by using Asp. niger for sugar concentration, inoculum volume and urea were 2, 15 and 0.28%. While the best inoculum volume were 10% and urea concentration, 0.07% by using hydrolyzed peas peel cake as a medium for produced single cell protein.

3. Amino acid composition and nutritive evaluation of single cell protein produced by fungi:

Mosmar (1975) reported that the evaluation of amino acids of the fungal mycellia Spicaria elegans (1), Cladosporium sp. (2), C. cladosporioides (3), Dactylium denderoides (4), Linderina penssispora (5) and Heterocephalum aurantiacum (6), showed that all investigated cultures contained all the essential amino acids except threonine. However, D. denderoides and L. pennispora contained the highest amounts of the ten essential amino acids in comparison with casein.

El-Shimi (1977) found that the fungal mycelia of Cladosporium cladosporioides, C. sp. and Spicaria elegans contained 12 amino acid, of which 6 are essential. The author, also observed that the sulphur containing amino acids and isoleucine were deficient in all investigated strains. The tryptophan in S. elegans and C. cladosporioides was also deficient.

Yashida and Hoshii (1980) found that 5 SCP samples from 16 samples produced from Aspergillus tamarii, Asp. oryzae, Candida utilis, Pseudomonas sp. and Alteromonas thalassomethanolica had excellent nutritive value, comparable to single cell protein available commercially in Europe. The authors added that all samples were palatable to the chicks and no sign of acute toxicity was observed.

The mycelia from Phanerochaete chrysosporium grown in vinasse contained about 23% protein which is rich in lysine. Methionine was the limiting amino acid as mentioned by Cardoso and Nicoli (1981).

Abd-Allah (1981) found that the essential amino acids, valine, phenylalanine and methionine were higher in the mycelia of Penicillium sp. (6) compared with the FAO pattern, while the mycelia of Rhizopus sp. (35) showed higher content of lysine, valine and phenylalanine.

4. Production of SCP by *Saccharomyces cerevisiae*:

Tirmazi et al. (1973) found that the maximum yield (46.1%) of *Saccharomyces cerevisiae* IMI-39916 was obtained when the mash contained 1.0% total sugars, 20 mg P/g of sugar and 30 mg N/g sugar. Change in pH between 4.0 and 5.0 did not affect the yield significantly. The molasses used contained reducing sugars of 17.82%, non reducing sugars 49.26%, total N 0.75% plus 1876 ppm of P, on dry weight basis.

Beschkov et al. (1979) mentioned that *S. cerevisiae* 0578, batch cultured at 28-29°C for 24 hr on molasses medium containing 0.192% sugar and amended with 400 mg $(\text{NH}_4)_2\text{SO}_4$, NH_2CONH_2 , $\text{NH}_2\text{H}_2\text{PO}_4$, $(\text{NH}_4)_2\text{HPO}_4$, NH_4Cl or NH_4NO_3 3/1. gave 3.60-4.81 g yeast solids, contained 35.5-47.2% pure protein and had protein to nucleic acid ratio of 6.4-6.38. $(\text{NH}_4)_2\text{H}_2\text{PO}_4$ was the best N source.

A *Saccharomyces cerevisiae* strain with high protein formation activity was cultured on onion juice with $(\text{NH}_4)_2\text{HPO}_4$ added as N source. The protein yield, content of several amino acids and carbohydrates were higher in the yeast grown on onion juice than the yeast grown on synthetic medium (Ghonaim et al., 1980).

Dosanjas et al. (1980) added $(\text{NH}_4)_2\text{SO}_4$ and urea to the medium and found that the protein production was maximum

when the N was added to the medium in the amount of 0.6 g/l. They mentioned also that urea was economically more attractive than $(\text{NH}_4)_2\text{SO}_4$.

Abd El-Lattef (1987) found that the best growth of Saccharomyces cerevisiae shown at the organic matter of urea at 48 and 72 hr. The highest protein content (50%) was shown after 72 hr of incubation in all yeast strains used.

Madi et al. (1975) used Saccharomyces cerevisiae for fermentation of Egyptian black strap molasses medium. Analysis of fooder yeast showed that it contained 95.0% dry matter, 42.0% protein, 5 pantothenic acid, and folic acid 25 mg/kg, Fe, Mn, Zn and Cu were found in the amounts of 280, 95, 200 and 50 mg/100 g, respectively.

Kellems et al. (1981) evaluated single cell protein (SCP) samples collected from several pulp mills in the Pacific North West by laboratory procedure. Amino acid analysis showed that SCP was higher in methionine than cottonseed meal and had a similar level of lysine. True protein ranged from 51.6 to 65.9% of the crude protein. Pepsin digestibility of crude protein ranged from 16.2 to 36.8%. Protein digestibility was increased by 6.3 to 11.3% when SCP was incubated in a buffered rumen fluid for 24 hr. The range of mineral composition was: P (0.62-1.55%),

Ca (0.14-0.99%), K(0.27-5.52%), Mg (0.07-0.59%). The concentration of trace minerals and heavy metals varied considerably from sample to another.

Abd El-Lattef (1987) found that the chemical composition of Saccharomyces cerevisiae produced from grown on standardized cane molasses media for crude protein, ether extract, ash and carbohydrates were 46.0, 0.50, 8.93 and 43.40% (on dry weight basis), respectively. The amino acid pattern, of all strains of protein makes yeast strains, was of a good nutritive quality. The content of essential amino acid of the different yeast strains was composed with the same essential amino acid of FAO reference pattern for hypothetical high biological value protein. Methionine and tryptophane were the limiting amino acids in all yeast strains under study.

5. Nucleic acid content of single cell protein:

Rolfe and Spicer (1973) reported that the nucleic acid content of a cell is dependent on its rate of growth, a consequence of the fact that nucleic acids are involved in the synthesis of proteins. Microbial cells are the favourable environment provided in a fermentor to grow and multiply rapidly and their content of nucleic acid may be up to 15% (on dry weight basis), which is much greater than occurs in protein foods of animal origin such as meat or fish. Nucleic acids after ingestion give rise to uric

acid in the blood and because of low solubility and possibility of precipitation may give rise to disorders similar to gout. Accordingly the Protein Advisory Group (PAG) (1970) have recommended that the ingestion of nucleic acid from such sources should not exceed 2 g/day. If microbial protein is to be used as a major source of protein in the diet its nucleic acid content must be reduced by some suitable treatment.

Schellart (1975) reported that the nucleic acid content of different microorganisms depends on both growth rate and growth conditions. Furthermore, the nucleic acid content would also differ according to the specific organisms under study.

Tannenbaum and Pace (1976) reported that the RNA is really the problem, because DNA is at a low concentration. The problem of nucleic acid levels in general is a problem which is related primarily to usage of the materials in fairly high concentrations of foods. If you want to use materials as a food additive let us say, 3-5% concentrations of nucleic acids, the amount being added to the diet would be so small as not to be of any significance, but if one want to consider using single cell protein directly as a human food in large amounts, this will be then a problem associated in getting rid of the RNA. It has not been a problem to develop processes which will

reduce RNA. It has been a problem to develop processes which are as economical as one would like to reduce RNA and this is a general problem with single cell protein production and not one that is specifically related to the question of food from waste.

Worgan (1976) found that the nucleic acid content for Aspergillus niger, Fusarium semitectum, Gliocladium deliquescens and Trichoderma viride were 3.4, 3.2, 3.9 and 4.7 g/100 g mycelium. The nucleic acid content of the mycelium of all four species is significantly lower than that reported for food yeast (10 g per 100 g cells). Although the content of nucleic acid is a function of growth rate, it has been found that F. semitectum mycelium does not contain more than 5% nucleic acid when it is grown at the same growth rate as yeast, namely at a mean protein doubling time of less than 5 hr.

Mourad (1985) found that the nucleic acid content of both Myrothecium verrucaria and Trichoderma viride grown on pretreated beet pulp was found to be less than 1% (calculated per 100 g dry weight of biomass).

Abd El-Lattef (1987) found that the nucleic acids content for Saccharomyces cerevisiae, Candida intermedia and Pichia rodanensis cells were 7.16, 8.15 and 8.06%, respectively. RNA composed about 93-96% of total nucleic acids while DNA was about 3.8-6.5%.

D. Pectin:

Pectic substances are mainly deposited in the primary cell wall during the early stages of growth when the area of the wall is increasing. Meristematic and parenchymatous tissues are therefore particularly rich in pectic substances. They occur predominantly in water-insoluble form which is called proto-pectin. The texture of fruits and vegetables on growing, ripening and storage is strongly influenced by the amount and nature of pectin present, (Voragen and Pilnik, 1970). They added that pectins form gels under certain circumstances and this property has made them very important food additives for jam, jelly and marmalade as well as for confectionery industry. Pectin is also used in medical preparations because of its pharmacological additives. New applications developed by using pectin to help the drying of fruit powders, and using low ester pectin for osmotic drying. Pectin also may be used as an effective synergistic antioxidant.

The most important factor in the production of pectins is the suitability of raw material sources. Pectins are widely applied as gelling, thickening or stabilizing agents (Pilnik and Voragen, 1980). Two main types of pectin gels exist: calcium pectate gels containing low methoxyl pectins (Weiss, 1979), and sugar-acid-pectin gels containing high methoxyl pectins. In fruit juice technology, pectin

and pectolytic enzymes play an important role (Rambouts and Pilnik, 1978).

Pectin has a nutritional function as dietary fiber component (Bock and Kraus, 1978; Cumming et al., 1979; Jenkins, 1980 and Stasse-Wolthius, 1980).

1. Molecular structure of pectic substances:

Pectin is not a homopolysaccharide but it has a chain structure of axial-axial α -(1-4) linked D-galacturonic acid units containing blocks of L-rhamnose rich regions with mainly arabinose, galactose and xylose as side chains.

Aspinall et al. (1968) reported that many pectic substances in which D-galacturonic acid is the principal constituent containing neutral sugars, especially D-galactose, L-arabinose and L-rhamnose.

Neukom (1980) demonstrated the new insights into the structure of pectic substances. They discussed the present theories on the structure of pectin and protopectin.

De Varies (1981) stated that the pectin molecule contains rhamnose, arabinose, xylose, galactose, glucose and galacturonic acid residues. No mannose could be detected. The neutral sugar component of the glycans bound to the galacturonan was formed contained, except for the relative amounts of galactose.

Klavons and Bennett (1987) studied the nature of the pectin constituent of commercial lemon juice cloud. They found that a small amount of the cloud pectin was classified as protopectin. The remainder of the cloud pectin was mainly in the form of calcium pectate.

2. Sources of pectin over the world:

The main raw material sources of commercial pectin over the world are still only, apple pomace obtained from cider processes and citrus peels left over the pressing of citrus juices. A new sources of raw material have been suggested recently.

Tresslar and Joslyn (1971) noted that percentage of total pectic substances in lemon peel, valencia orange peel, apple pomace, carrot pomace and beets were about 35.5, 28.8, 17.6, 18.6 and 12.0% on dry weight basis, respectively.

Kim et al. (1978a) found that the head and stalk of sunflower contain 15-24% and 4-7% pectin, respectively, depending on cultivars and stages of maturity.

El-Atawy (1984) stated that pectin content varied from 26.79 to 29.46 and from 23.10 to 27.70% in garlic skin and dry garlic foliage, respectively. The total pectic substances in beet pulp and mango peels were 18.31 and 23.45%, respectively.

3. Production of pectin:

3.1. Pretreatment of raw material:

Sarhan (1975) used ethyl alcohol 80% for 20 minutes to remove all possible interfering compounds in onion foliage and pigmented skins before extracting the pectic substances, then cooling and filtration. This process was repeated twice using a fresh alcohol solution each time.

Campbell et al. (1978) mentioned that, pectinases were inactivated by stirring samples of ground sunflower stalks and heads in 80% aqueous ethanol (1 : 20 W/V) at 90°C for 15 minutes. The suspensions were centrifuged, the supernatants were discarded and the ethanol insoluble residues were dried for pectin extraction.

Kim et al. (1978b) and Lin et al. (1978) mentioned that prior to pectin extraction, sunflower head samples were washed with hot water (75°C) for 10-15 minutes to remove soluble pectins and pigments.

Trehan and Ahmed (1947) found that, extracted pectin from lemon rinds by refluxing for 6 hours with 0.0133 N hydrochloric acid, 0.5% ammonium oxalate, or hot water and found that the first extracting agent gave highest yield and purity.

extraction with oxalic acid 0.75%, citric acid 2.0%, tartaric acid 4.0% and hydrochloric acid 0.05 N for beet pulp. Further increase in acids produced slight higher yield but lower quality pectin while low concentration of acids was not efficient in extracting most of pectin present in beet pulp. He showed that the yield of pectin increased by increasing extraction temperature and solvent concentration while time of extraction was maintained constant. Moreover, the author added that the high yield of good quality pectin was obtained from garlic wastes by extraction with ammonium oxalate solution rather than acid's extraction.

3.2. Precipitation and purification of pectin:

Joslyn and Deuel (1963) indicated that alcohol precipitation from aqueous solutions of pectic substances could be carried out by gradual addition of alcohol to the extract until a final concentration of alcohol of 50-53% by volume was reached. Hydrochloric acid was also added to improve the yield and the purity of the alcohol precipitate.

Voragen and Pilnik (1970) stated that pectin with high purity was attained by precipitation with acidified and diluted alcohol, dialysis, electro dialysis or ion-exchange.

Amer et al. (1972a) studied the effect of successive precipitations with acidified alcohol on the purity of pectin extracted from Citrullis vulgaris. They noted that the purity of pectin separated after the first, second and third precipitation was; 72, 85.3 and 96%, respectively. The pectin percentage was 1.32%.

El-Atawy (1984) reported that precipitation of garlic wastes pectin with acidified alcohol increased the yield of anhydrous galacturonic acid (A.G.A.) than that precipitated with non-acidified alcohol. However, precipitation with non-acidified alcohol increased moisture, ash and flow time. Garlic waste pectin, precipitated with non-acidified alcohol seemed to be slightly higher in its methoxyl and acetyl contents than that precipitated with acidified alcohol. He also mentioned that the purification process affected the properties of beet pectin extracted with different acids which decreased, moisture and ash besides an increase in A.G.A. and reducing power.

4. Evaluation of pectin:

4.1. Chemical properties:

4.1.1. Moisture content:

Moisture content of pectin, as recommended by the FAO (1978), should not be more than 12%.

Sarhan (1975) found that the moisture content of skin and dry foliage onion pectin ranged from 9% to 12%.

Sosulski et al. (1978) mentioned that the ash content of sunflower pectin ranged from 3.2 to 4.0%, while in citrus pectin it ranged from 1.3 to 3.2%.

El-Atway (1984) reported that the ash content of "Balady" garlic foliage and mango peel pectin extracted by ammonium oxalate and precipitated with acidified alcohol varied from 1.82 to 5.98 and 0.92 to 1.77%, respectively. He also found that the ash content of beet pulp pectin extracted by oxalic acid, citric acid, tartaric acid and hydrochloric acid ranged from 1.03-7.53, 1.53-4.93, 1.39-1.6 and 7.20-13.7%, respectively.

4.1.3. Methoxyl content:

Kertesz (1951) analysed commercial pectins isolated from the principle natural sources. He found that the percentage of methoxyl content varied from 6.4 to 10.8%. He noticed that the ester groups influenced the solubility of the pectin. The solubility in water was increased with a greater proportion of methyl ester. The methoxyl content varied even in the same raw material. This observation was due to the degree of demethylation which occurred during extraction and purification of the pectin.

Raunhardt and Neukom (1964) supported the method of Myers and Baker (1929) who determined the free carboxyl groups by direct titration with alkali and the methoxyl groups were determined by saponification with alkali.

Sarhan (1975) determined the methoxyl content of pectin extracted from the pigmented onion skins and the dry onion foliage. He found that it ranged from 5.75 to 6.44% and 6.21 to 6.77%, respectively.

Gorin and Kotenko (1982) mentioned that the degree of methoxylation of the pectin derived from apples, beets, and citrus fruits ranged from 32 to 80%.

Turmucin et al. (1983) found that the methoxyl content of sunflower head pectin was 1.86%.

El-Atawy (1984) reported that the methoxyl content of "Balady" garlic foliage and mango peel pectin extracted by ammonium oxalate and precipitated with acidified alcohol ranged from 0.54 to 0.61% and from 0.40 to 0.44%, respectively. He stated that the acetyl content of beet pulp pectin extracted by different acids varied between 1.89 and 3.86%.

4.1.4. Acetyl content:

The importance of acetyl determination was detected from the results reported by Pippen et al. (1950) on acetylated citrus pectin. They found that preparations containing 3.5-5.2% acetyl failed to form sugar-acid-pectin jellies, while upon removal of the acetyl groups jelly was prepared from those preparations in the usual manner. They also

noted that the introduction of acetyl groups would reduce the solubility of pectinic acids in water. They concluded that "the presence of acetyl groups prevents jelly formation".

McComb and McCready (1957) determined the acetyl content of pectin by colorimetric method based upon the reaction of acetate with alkali hydroxylamine to form acetohydroxamic acid which reacts with ferric ions to form a soluble red iron complex. They found that lemon peel pectin, apple pectin and tomato pectin contained, 0.37, 0.45 and 0.79% acetyl, respectively.

Pippen et al. (1950) determined the acetyl content of pectin by saponification of acetyl groups with sodium hydroxide then distillation. They observed that the method was rapid, economical of material, required few operations and was accurate.

4.1.5. Reducing power:

Sarhan (1975) found that increasing the extraction temperature increased the degree of hydrolysis and hence reducing power. He also mentioned that the precipitation of pectin with acidified alcohol increased the reducing power. He stated that the reducing number of pectin samples extracted from skins and dry onion foliage ranged from 3.05 to 6.37.

El-Atawy (1984) mentioned that the reducing power of "Balady" garlic foliage, mango peel and beet pulp pectin ranged between 3.17-4.20, 2.98-3.75 and 2.78-3.91, respectively.

4.2. Physical properties:

4.2.1. Optical rotation:

Hirst (1942) reported a specific optical rotation ranging from 170° to 230° for polyglacturonic acids from different sources.

Kertesz (1951) showed that the specific rotation of highly purified citrus pectinic acids ranged from +237° to +250°. He added that, araban which often accompanies pectic substances in plant tissues and preparations was strongly levorotatory ($(\alpha)_D^{20} = -123^\circ$ to -167° , while pectinic acids were dextrorotatory. Therefore, the presence of even small proportions of arabans had a considerable effect on the observed optical rotation of pectinic acid preparations.

Abdel-Fattah and Edrees (1971) found that the specific rotation of pectic substances isolated from pigmented onion skins ranged from +121° to +291.2°.

Sarhan (1975) reported that the $(\alpha)_D^{20}$ of pectins of pigmented skins and dry foliage onion varied between +235° and +305°.

Nour (1977) noted that the specific optical rotation of pectin isolated from mango fruit varied from $+210^\circ$ to $+212^\circ$, while commercial citrus pectin had $+217^\circ$. Also, he mentioned that consequence of the configuration of the glycosidic linkage was the high positive optical rotation of pectin e.g. the pectin extracted from strawberry showed $+251^\circ$ in water, and apple pectin $+230^\circ$ and beet pectin $+196^\circ$.

El-Atawy (1984) found that the optical rotation of pectin extracted from garlic (foliage and skins), mango peels and beet pulp ranged from $+200.80^\circ$ to $+229.01^\circ$, from $+180.40^\circ$ to $+270.70^\circ$ and from 149.40° to 175.40° , respectively.

4.2.2. Molecular weight:

Ranhardt and Neukom (1964) concluded that the strength of pectin-sugar-acid-water-jelly depends mostly on molecular weight and slightly on other pectin properties.

Neukom (1967) reported that the molecular weights of commercial apple, lemon and beet pectins calculated from viscosity measurements were 67,000, 89,000 and 62,000, respectively.

The molecular weight of low methoxyl pectin extracted from pigmented skins and dry foliage of onion ranged from 40,000 to 49,150 and 41,700 to 45,110, respectively (Sarhan, 1975).

Nogoibaeva et al. (1980) reported that the molecular weight of beet pectin determined by ultracentrifuging and gel filtration was ~18,000.

Turmucin et al. (1983) mentioned that the molecular weight of the sunflower head pectin was 27,300.

El-Atawy (1984) stated that the molecular weight of pectin extracted from garlic (foliage and skins), mango peel and beet pulp varied between 43617-84042, 57446-114893 and 57446-85106, respectively.

Absera and Shelukhina (1984) studied beet pulp pectin by gel-filtration. They found that molecular weight was in the range of 5,000 to 40,000.

Michel et al. (1985) found that molecular weight of beet pulp pectin was 30,000.

4.2.3. Pectin gels and jelly grade:

Rouse and Knorr (1970 and 1971) reported that the purity of pectins from lemon peel and pulp ranged from 75.2 to 87.0% and from 65 to 92%, respectively. On the other hand, they found that the jelly grade of pectins from lemon peel ranged from 225 to 272 grade and from 166 to 285 grade for pectine from lemon pulp. They stated that the methoxyl content of pectins from lemon peel and pulp was 12.0 and 12.3%, respectively.

Sarhan (1975) stated that the jelly grade of pectins extracted from pigmented skins and dry foliage onion ranged from 250 to 446.

The pectic substances which produce firm jellies are relatively high molecular weight molecules with a relatively high percentage of methyl ester groups. When a hot aqueous mixture of sugar, acid, and pectins is cooled, it sets into a gel. With very low pH's, the amount of pectin can be decreased and a satisfactory gel still formed. An optimum pH is usually found; and if the pH is lowered below this point, the firmness of the jelly diminished and excessive syneresis develops. Low ester pectins are produced by de-esterification with either acid, enzymes, or alkali. The products formed from these three methods differ sharply in their physical characteristics and particularly in their reaction to divalent ions (Meyer, 1978).

Blanshard and Mitchell (1979) reported that the pectins can form two types of gels depending upon their degree of esterification. High methoxyl pectins will form gels at acid pH 5.0 and in the presence of a high concentration of sugar. Low methoxyl pectins are similar to alginates in gelling properties, and require a divalent cation such as calcium, in order to form gel. These gels could be formed without sugar and over a wide pH range. Low methoxyl pectins have a degree of esterification in the 20-40 range.

Srirangaragian and Shrikhande (1979) found that the jelly grade of pectins extracted from Dassehri, Langra and Alphonso mango peel were 155, 175 and 200, respectively. They added that the jelly grade of apple pomace and orange pectins were 200 and 150, respectively.

Alexander and Sulebele (1980) mentioned that jelly grades of lime, orange, sweet orange and grape fruit pectins were 225, 209, 180 and 200 grade, respectively.

El-Atawy (1984) reported that the jelly grade of pectins isolated from garlic (foliage and skins), mango peel and beet pulp ranged from 244-over 270, 146-226 and 88-202, respectively.

Michel et al. (1985) extracted pectins from sugar beet pulp on a laboratory scale and mentioned that pectins physicochemical properties confirm their poor gelling ability.