Sudan University of Science and Technology

College of Graduate Studies

Extraction and Assessment of Pectin from Pumpkin Peels

استخلاص وتقييم جودة بكتين قشور القرع

BY

Awadia Aldow Ramadan Hamed

B.sc. (Agric) Honoures

Sudan University of Science and Technology (2008)

Adissertation Submitted to Sudan University of Science and Technology in partial fulfillment for the Requirements for the Degree of Master of Science in Food Science and Technology

Supervisor

Dr. Salma Elghali Mustafa

Department of Food Science and Technology

College of Agricultural Studies

Sudan University 0f Science and Technology

October 2015

DEDICATION

For every brilliant sun that transfer the darkness to light

My dear mother .

For every moon who never sleep to offer us good life all the day and night

My dear father.

For every stars when I see them I feel hop

My dear brothers and sisters

For every diamond when I work with them I feel safe

My dear friends.

For my best supervisor ever the whole devotion and respect.

For all the people who have helped me the fluent of what said

ACKNOWLEGEMENT

I wish to thank my God who is more than generous with me expertise and precious time.

A special thanks to Dr. Selma Algali for her countless hours of reflecting, reading and encouraging.

I would like to acknowledge and thank the staff members of the department of food science and technology /Sudan University of Science and Technology for their continued support.

Also I would like to thank Ustaz Khalid Department of Animal production University of Khartoum for his patience and helps to conduct my research.

TABLE OF CONTENT

Title **Page No**

The extracted pectin from pumpkin peels in this study was 6.24% by using nitric acid and 5.36% by using citric acid .The study showed that nitric acid produced higher than citric acid respectively as shown in figure 4.1. Significant difference p0.05 was observed between two acids. The result was higher compared with the range of 0.253-0.233 % for pectin extraction from alcohol insoluble solids from pumpkin peel were reported as the use of hydrochloric acid

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DE: Degree of Esterification

DA: Degree of Amidation

MeO: Methoxyle Content

HM: High Methoxyl

LM: Low Methoxyle

EDTA: Ethylen Diamine Tetracetic Acid

ADI: Acceptable Daily Intake

JECFA : Joint Experts Committee for Food Codex Alimentarius

SCF: Scientific Committee for Food

MW: Molecular Weight

Gal A: Galacturonic Acid

ABSTRACT

Pectin is defined as complex mixtures of polysaccharides that make up approximately one third of the cell-wall dry substance of most types plants. This study was carried out to extract pectin from fully ripen pumpkin (*Cucurbita spp*.) using two different methods; soxhlet acid extraction method and acid extraction method. And to study the influence of using different acids on the yield of pectin; nitric and citric acids were used. Moreover, to examine the effect of time in pectin yield, the extraction process was conducted using different times 30, 60 and 90 min. The chemical compositions of pumpkin peel (moisture, ash, protein, fat, fiber, total sugar and calcium) and the chemical properties of pectin extracted with both nitric and citric acids (methoxyl content, acetyl content, equivalent weight and degree of esterification) were also detected. The results obtained for the soxhlet acid extraction method and acid extraction method were (7.72% Nitric acid and 6.80 % citric acid) and (6.24% nitric acid and 5.36% citric acid) respectively which indicated that the use of soxhlet acid extraction method with nitric acid obtained the highest yield of pectin. Also it was found that, the maximum time for pectin extraction was 60 min.

The result for the chemical compositions of pumpkin peel (moisture, ash, protein, fiber, fats, total sugar and calcium) were (20.1, 7.1, 3.2, 10.15, 2.3, 57.15 and 0.308 %) respectively. The study found that the chemical properties of pectin extracted with both nitric and citric acids were; for equivalent weight (1250 and1250 g/mol), methoxyl content (6.20 and 6.29%), acetyl contain (0.43 and 0.43%) and the degree of esterification (66.53 and 66.57%) respectively. The result obtained from this study indicated that pectin extracted from pumpkin peel is with high quantity and quality and is promising for commercial production.

خلاصة الأطروحة

يعرف البكتين كخليط معقد من السكريات المعقدة التي تكون ثلث جدر الخلايا الجافة تقريبا في معظم أنواع النباتات. اجريت الدراسة علي نوع من القرع المحلي لاستخلاص البكتين من قشور القرع وتحديد الطريقة المثلي التي تعطي اعلي نسبة للبكتين استخدمت طريقة الاستخلاص بالحمض و طريقة الاستخلاص بالحمض مع استخدام السوكسلت. ولتحديد نوع الحمض الذي يعطي اعلي إنتاجية من البكتين استخدم حمض النتريك وحمض الستريك. ولمعرفة تأثير عامل الزمن علي نسبة البكتين تم بالاستخلاص علي فترات زمنية مختلفة (30, 60 و90 دقيقة ولتحديد التركيب الكيميائي لقشور القرع تم تقدير (الرطوبة, الرماد, البروتين, الدهون, الألياف, الكاربوهيدرات والكالسيوم).و لتقييم خواص البكتين المتحصل عليه تم اختبار الوزن المكافى والمحتوي الميزوكسيلي والمحتوي الاستيلي ودرجة الاسترة. اظهرت الدراسة أن استخدام طريقة السوكسلت مع الحمض أعطى نسبة عالية من البكتين(7.72% حمض النتريك و 6.8 % حمض الستريك) اعلي من استخدام الحمض دون السوكسلت الذي أعطى (6.24 % حمض النتريك و5.36% حمض الستريك). كما أن استخدام حمض النتريك اعطي نسبة عالية من البكتين اعلي من استخدام حمض الستريك . وأظهرت الدراسة أن هناك زيادة في نسبة استخلاص البكتين بين (30-60) دقيقة و ليس هناك فروق معنوية في استخلاص الببكتين بعد 60 دقيقة وبذلك تعتبر60 دقيقة هي الزمن الأمثل لاستخلاص البكتين. وجدت الدراسة ان نتائج التركيب الكيميائي الرطوبة, الرماد, البروتين, الدهون, الألياف, الكاربوهيدرات والكالسيوم كالآتي علي التوالي20.1, 7.1, 3.2, 2.3, 10.15 57.15و0.308 % ونتائج خصائص البكتين المستخلص باستخدام حمض النتريك وحمض الستريك يحتوي الآتي علي التوالي الوزن المكافئ 1250, 1250%جم/مول والمحتوي الميزوكسيلي 6.20,7.23 % والمحتوي الاستيلي43, 0.43 % ودرجة الاسترة. 66.53, 66.57 % . كما أوضحت النتائج المتحصل عليها من هذه الدراسة أن كمية ونوعية البكتين تعد بإمكانية إنتاج البكتين من قشور القرع علي المستوي التجاري

CHAPTER ONE

INTRODUCTION

Pumpkins belong to gourd family *Cucurbitceae* which also contain water melons, cuscumbers, gourds, marrow and squash (Elshafie, 1981). *Cucurbits* consist of 110 genera and 640 species predominant in the tropics mostly as herbs of very rapid growth and climbing habit with abundant sap (Treas and Events 1989). Pumpkin is rich in polysaccharides, carotene, mineral salts, vitamins, and other substances beneficial to health, resulting in various processed food products being developed (Nutr, 2006 and Zhemerichkin; Ptichkina, 1998).

Usually fruits are processed into juice, beverage, squash and syrups. During processing, peel is contributes almost 5-20 % of the total fruit. Therefore, the huge amount of by-products (55- 60% peel and 5-10% seed) has result in pollution of the environment. However by-products can be used as functional food ingredients such as photochemical, pharmaceuticals, food products, essential oils, seed oil, pectin and dietary fibers. Therefore by-products are considered to be rich sources of edible and heath promoting agents (Ali, 2014).

Pumpkin is also a low cost source of pectin (Murkovic *et al*., 2002). Pectin is a multifunctional abundant component from cell walls of all plants (Ngouémazong *et al*., 2012; Willats *et al*., 2006). Pectin is polysaccharides consist mostly of polymers rich in galacturonic acid, containing significant amounts of rhamnose, arabinose and galactose as well as 13 other different monosaccharides (Vincken *et al.*, 2003). Waldron et al., (2003) reported that, the composition, structure, and physiological properties of pectin can be influenced by extraction conditions as well as source, location, and many other environmental factors. Therefore the network of pectin must be broken to be extracted.

Pectins are typically extracted from citrus fruits and apple pomace and it traditionally used as a gelling agent for jellies and marmalades. In combination with water and some other substances, it can act as a thickener, gelling agent, stabilizer, emulsifier, cation-binding agent, etc. (Bottger, 1990). Once substance having so many properties of technological interest, this makes pectin a biopolymer especially valuable for medicine, food production as well as for applications in drug encapsulation (Benjamin *et al*., 2012; Souza *et al.,* 2009; Ptichkina *et al.,* 2008).

The problem statement:

Pumpkin is cooked in many food but the peels are thrown away as waste.

The main objective of study:

The main objective of this study is to extract and evaluate pectin from pumpkin peel.

The specific objectives of study:

1. To determine chemical composition of pumpkin peels (moisture, protein, ash, fat, fiber, total sugar and calcium)

2. To investigate the effect different extraction methods (soxhlet acid extraction method and acid extraction method) with different acids (nitric and citric acids) in yield of pectin.

3. To examine the effect of time on pectin yield.

4. To evaluate the properties of the extracted pectin.

CHAPTER TWO

LITERURE REVIEW

2.1 Pumpkin:

2.1.1 Background:

The fruits of pumpkin (*Cucurbita moschata*) have been widely accepted as a dietary constituent among peasants in China; it is green when unripe and turns yellow on ripening. Pumpkin has received considerable attention in recent year because of nutritional and health protective value of the protein and oil from seeds as well as the polysaccharides from the fruits (Barbara and Murkovic, 2004; Murkovic *et el*., 2004).

2.1.2 Production of pumpkin in Sudan:

In Sudan *Cucurbita* SPP (pumpkin) is planted in February – March – and Augest – October, with 1-2 kg /fed seed rate , on one side of the mustaba 2-3 meter wide and one meter between plants .After 12-15 weeks fruits are ready to harvest with average yield 150-200 kg/fed (Ibrahim, 2008). El Shafie (1981) reported that most of pumpkin consumed in Sudan is grow in Kordofan (Western Sudan) where it contributes up to 39.1% (12,174 metric tons in the year 1980/81.

2.1.3 Chemical compositions of pumpkin:

Sudanese pumpkin fruits evaluated by El Awad (1980) found to contain moisture 89.5%, protein 1.3%, fat 0.1%, ash 0.5%, carbohydrate 8.6%, calcium 22mg, iron 0.5mg, food energy of 41(kcal) 37mg, on fresh weight basis . While El Shafie (1981) reported that pumpkin fruits obtained from three growing areas in Sudan contains moisture range of 92.80-93.95%, protein 0.51-0.70%, ash 1.08-1.46% , crude fiber 0.51-0.85%, fat

0.17-0.22%, carbohydrate 3.65-4.62% , alcohol insoluble solids1.97-3 2.59% , total solids 4.05-7.19 %, pH 5.85- 6.00, total titrable acidity 0.036-042%, ascorbic acid 0.018%, reducing sugar 2.67%, total sugar 1.13 -2.69 % and total pectin 0.56% on fresh weight basis.

2.1.4 Uses of pumpkin fruits:

The most important use of tropical pumpkin is consumption as mature vegetable fruit, normally cooked and eaten as pudding or snack. Fruit size, internal flesh color and thickness, soluble solids and, in some cases fruit skin color, have been found to be subject to consumer preference (Carbonell *et al.*, 1990; Maynard, 1996). Recent studies have examined the application of pumpkin powder to enhance the loaf volume and organoleptic acceptability of wheat bread (Ptitchkina *et al.*, 1998). Besides that, in Sudan it has a good market price and could be shipped for long distances with little damage and most of pumpkin fruits are consumed locally when cooked with meat and tomato (Obeidalla and Riley, 1984). Moreover, in Sudan pulp from pumpkin fruit is used in the production of jam in limited amount, El shafie (1981) carried out a research on the pectic substance from the fruit pulp which found that it is a good source of pectin for jam making industries.

2.1.5 Chemical compositions of pumpkin peel:

Chemical compositions of pumpkin peel are Moisture 84.7- 90.26%, Ash 0.81- 1.35 %, protein 1.64- 1.3 %, crude fiber 3.21- 4.82 %, fat 0.04- 0.057 %, carbohydrate 4.05 -5.17% , total soluble solid 2.14-2.89 %, alcohol insoluble solid 5.75-11.51%, pH 5.60-5.53, total titrable acidity 0.018-0.029%, ascorbic acid 0.005-0.004%, reducing sugars 2.88-3.04%, total sugars 3.33-4.85%, magnesium 0.005-0.006%, calcium 0.062-0.09% (Ibrahim, 2008)

2.2 Pectin:

2.2.1 Definition of pectin:

Pectin is defined as complex mixtures of polysaccharides that make up approximately one third of the cell-wall dry substance for most types of plants (Van Buren, 1991). It is presents in all the higher plants and occurs in the intercellular or middle lamella region, and provides the channels for passage of nutrients and water. In cell walls, pectic substances serve as one of the main agents cementing the cellulose fibrils and many of them are linked covalently to other polymers (Inari *et al*., 2002).

2.2.2 Chemical nature of pectin:

Pectin is primarily made up of D-galacturonic acid joined by α-1, 4 glycosidic linkages (Van Buren, 1991). As a part of the plant structure, pectin is a complex mixture of blocks of homogalacturonic acid called ‘smooth regions’ mixed with blocks of homogalaturonic acid containing many neutral sugars including rhamnose, galactose, arabinose, xylose, and glucose called ‘hairy regions’ (IPPA, 2001).

2.2.3 Classification of pectin:

After extraction of pectin, it consists of smooth galacturonic acid regions with a few neutral sugars still attached (IPPA, 2001). Extracted pectin can be categorized into two major categories depending on the percentage of galacturonic acid residues that are esterified with methanol. When the degree of methoxylation (DM) is greater than 50% the pectin is considered as high methoxyl pectin, and when the DM is below 50% it is considered low methoxyl pectin (Braddock,1999).

2.2.3.1 High methoxyl pectin:

Pornsak (2003) reported that, high methoxyl pectin HM has relatively high portion of the carboxyl group as methyl esters greater than 50 %. High-methoxyl pectins form gels with the presence of a high concentration of co-dissolved sugar (> 55% w/w) and at an acidic pH (< 3.6) . The presence of the sugar as a cosolute decreases water activity and effectively dehydrates the pectin (Wang,2002). HM pectin can be divided into rapid set, medium rapid set and slow pectin depending on the setting time and temperature. Gel made with HM pectin have affirmed and short structure, are clear and with good flavor release. These gels are not heat reversible. Apart from the setting time, HM pectins are standardized with respect to grade, gel strength, setting temperature, viscosity and protein stability. It is suitable for high sugar jams, jellies and beverage.**(** Pornsak, 2003).

2.2. 3. 2 Amidated low methoxyl pectin:

Pornsak (2003) recorded that**,** amidated pectin is low methyl pectin (LM) obtained from high methoxyl pectin when ammonia is used in the alkaline de-esterification process. The degree of methoxylation (DA) determines the calcium reactivity of this pectin. LM form gels in the presence of a counter cation (most commonly calcium) with little or no co-solute sugar. This occurs within a wide pH range and with higher temperature stability, the lower the pectin DM the greater the tendency to precipitate in the presence of a cation, generally calcium. Calcium and d-orbital, divalent, transition metals (e.g. Cu2+, Co2+, Pb2+, etc.), use unfilled orbital’s to form coordination complexes with neutral and acidic carbohydrates. It is suggested that calcium’s radius (0.1 nm) is large enough to coordinate with the spatial arrangement of oxygen atoms in many sugars and can have extreme flexibility in the direction of its own coordinate bonds (Van Buren,1991).LM pectin form a spreadable, shear reversible gels in hot and cold conditions and is suitable for low sugar jams, fruit preparations and reversible jellies. The degree of amidation (DA) is expressed as a percentage of amidated galacturonic acid units to total galacturonic acid untits in the molecule of pectin (Pornsak, 2003).

2.2. 4 Properties of pectin:

Pectin dispersed in water is stabilized by hydrogen bonding and when sugar is added the stability is decreased by the dehydration action. At a sugar concentration of 50-65%, the dehydration is complete added hydrogen ions further upset the balance by reducing the negative charge on the pectin. Consequently, a gel forms as a result of an unsuccessful attempt at crystallization on the part of the aggregating pectin molecules. The gel forming capacity is described in terms of the jelly grade which is defined as the number of pounds of sucrose with which one pound of pectin will form a jelly of 65% total solids. Various methods and devices for measuring jelly firmness or strength have been developed (El shafie, 1981).

2.2.5 General Properties:

Venkey (2009) recorded general pectin properties as following:

1. Pectin is a solid powder, of white in color.
2. Pectin is practically odorless. It is mucilaginous.
3. Pectin is stable at ordinary conditions, becomes unstable in excess heat. Pectin is soluble in pure water, partially soluble in cold water. It is insoluble in alcohol and organic solvents. When pectin is mixed with alcohol or organic solvent and mixed with water it is soluble, whereas di- and tri- salts are weakly soluble or insoluble.
4. Pectin is combustible at higher temperatures, because of this it must be kept away from heat and any source of ignition.

5. Dry powdered pectin, when added to water, has a tendency to hydrate, very rapidly, forming gels.
6. Dilute solutions are newtonian, but at a moderate concentration, they exhibit non-newtonian, pseudo plastic behavior characteristics.
7. Viscosity, solubility and gelation are generally related (e.g. factors that increase gel strength will increase the tendency to gel, decrease solubility, and increase viscosity and vice versa).
8. Coulombic repulsion is present between the carboxyl ate anions which prevent the aggregation of the polymer chains.
9. Another property of pectin which plays a major role in most of its applications is its pH. When pH is lowered, ionization of carboxylate groups is suppressed, and this results in reduction in hydration of carboxylic acid groups. At higher pH the degree of methylation will be greater. At a pH of 5-6 low methoxylated pectin is stable, but high methoxylated pectin is stable only at room temperature.
10. Pectin does not undergo polymerization but undergoes depolymerisation. Depolymerisation occurs when pectin molecules are treated with dilute acids at higher temperatures. They break into smaller fragments. The pectin which is dissolved in solutions gets decomposed spontaneously by desertification as well as by depolymerisation. These rates depend upon the pH and on temperature. Desertification is favored by low pH. Maximum stability of pectin occurs only at pH 4.
11. The other properties which influence the wide spread applications of pectin are, degree of methoxylation, degree of esterification and degree of amidation.
12. Generally pectin is valued by the pectin grade, defined as number of pounds of sugar that one pound of pectin can carry in a jelly.

2.2.6 Extraction of pectin:

An enzyme extraction hydrolytic technology seams environmentally safe and more effective in terms of pectin yield (Ptichkina *et el.*, 2008). Enzyme and strong acid are also commercially used in pectin extraction (Whistler and Be Miller, 1997; Shkodina *et al*., 1998; Kalapathy and Proctor, 2001). The current technology of extraction is based on acidic hydrolysis and has at least two demerits. Acid extraction does not allow pectin to be extracted fully with no damage to its structure and it does not meet environmental safety due to acid usage.

Extraction by using various chemicals agents such as ethylendiamine tetracetic acid (EDTA), ammonium oxalate, sodium carbonate, sodium hydroxide and hydrochloric acid are generally been used for the fraction of pectin (Voragen *et el.*,1995). Commercially, pectins are extracted by treating the row material with hot dilute mineral acid, mainly nitric acid (Rolin, 2002). Laboratory scale extractions have been conducted to determine optimal pectin extraction conditions and the feasibility of pectin extraction from a number of different plant materials. The effects of temperature, time, and pH on pectin yield for orange pectin using nitric acid extraction was investigated by Aravantinos-Zafiris *et al.* (1991). Several acid can be utilized for the extraction of pectin according to(Hwang et. el., 1998; Dinu, 2001). The acids used for pectin extraction were the tartaric, malic, citric, lactic, acetic and phosphoric acids but was a tendency to use the cheaper mineral acids such as sulphuric, hydrochloric and nitric acids.

Optimal extraction conditions are for pH is 1.6, for temperature is 84 ºC and for time is 64 min resulted in yields up to nearly 26% of the dried peel weight. Galacturonic acid content, methoxyl content, and ash were reported to be independent of the extraction variables. Optimal extraction conditions found through varying extraction time, pH, and temperature for pectin extraction from sugar beet pulp were reported as the use of hydrochloric acid to adjust pH to 1.5 extracted for 4 hours at 80ºC (Phatak *et al.*, 1988). The resulting pectin yield was 19.53% dry basis at these extraction conditions. Extraction pH, time, and liquid to solid ratio were optimized in a study on pectin extraction from tropical fruits (Simpson *et al*., 1984). More over the resulting pectin yield was 0.253-0.233 % for pectin extraction from pumpkin peel were reported as the use of hydrochloric acid to adjust pH to 2 extracted for 60min at 80ºC (Ibrahim, 2008).

2.2.7 Uses of pectin in food industry:

Pectin is generally regarded as one of the safest and most acceptable of food additives and this is recognized by Acceptable Daily Intake (ADI) and the levels of ‘not specified’ by both the Joint Experts Committee for Food Codex Alimentarius (JECFA) and the Scientific Committee for Food (SCF) in the European Union (Schols and Voragen, 1998).

Pectin has many applications in food science, nutrition, cosmetics and pharmacy. It is widely used as food additives for thickening, gelling and emulsifying jams, soft, drinks, fish, meat products and milk (Barrera *et el.*,2002; Ralet *et el* .,2005). One of the well-known uses of pectin is in high sugar jams and confectionery jellies, dating back to at least the 18 th century (IPPA, 2001). Because it is a natural additive for foods, pectin is being considered for a number of applications beyond the traditional jams and jellies. Pectins are now used as thickeners, water binders and stabilizers.

It is used in yogurts and pastry glazes and as a stabilizer in drinkable yogurts and blends of milk and fruit juices (May, 1990). Pectin is also being used as a texturizing fat replacer to mimic the mouth-feel of lipids in low-calorie foods and shorter chain galacturonic acids have been considered as clarification agents in fruit juices (Braddock, 1999). Pectin has also been investigated for its use fullness in the pharmaceutical industry. Among other uses it has been considered in the class of dietary fibers known to have a positive effect on digestive processes and to help lower cholesterol (Braddock, 1999).

It also is utilized to stabilize liquid pharmaceutical emulsions and suspensions. Changes in pectic polymers have been described in many processes such as canning, lactic fermentation and heat treatment, but very little is known about change due to alkaline treatment as shown by Jimenez et el., (1996).

Pectin is not only necessary additive to form the structure of food products but also has medicinal benefits which include lowering the cholesterol level in blood, removing heavy ions from the body, stabilizing blood pressure and restoring intestinal function (Voragen *et el*., 1995). Also pectin fraction from the alcohol insoluble polysaccharide of pumpkin peels might help in improving gastro-intestinal health (Jun *et el*., 2006).

2.2.8 Raw materials for pectin production:

The world consumption of pectin constantly grows and has already exceeded 20.000 tons a year. Dried lemon or orange peel and apple pomace are the main raw material for its production (Phillips, 2000). All citrus contain pectin, and the richest sources are, lime lemon oranges and grapefruit (Bates, 2001). Searching for new pectin –containing raw materials is important task of science and industry (May, 1990).The main sources of pectin citrus peel, apple, apple pomace, Sisal flax and sun flower heads have been mentioned as other secondary source of pectin production. Ptickina (2000) reported that the pumpkin pulp seams more promising for pectin production in Russia.

2.2.9 Health and safety characteristics:

Pectin is a fine organic powder, and like flour, starch, and similar carbohydrate materials, has the potential to cause a dust explosion. It is therefore importantto observe good housekeeping practices such as collecting up spilled material, and minimising dusting by careful handling. A lesser but more common hazard is spillage of pectin solution or of powder pectin onto a wet floor, creating the risk of slips and falls. Pectin is not a particular environmental hazard, but has large spillages should be contained and disposed of carefully. Pectin is a component of the normal diet, and an approved food additive, and ingestion of pectin at reasonable levels is safe. As with any water-soluble gum, it is inadvisable to consume large amounts of dry pectin which could swell and possibly risk obstruction of the gullet.(Kertesz,1951 ;Schols and Voragen, 1998) .

2.2.10 Commercially Important Measurements:

Dixon (2008). Reported that, Years of pectin research have produced several important commercial analytical measures. While not all-inclusive, discussions of seven methods are included below:

Weight Average Molecular Weight MW:

is a very important physical property of pectin. It is the most important characteristic in determining the functional behavior of pectin. Gelling abilities of individual pectins are tied very closely with MW.

Total % Galacturonic Acid (%Gal A):

Gal A content, as with MW measurement, is important to the gelling capabilities of given pectin. For this measurement the polymer is degraded to monosaccharide via one of numerous chemical or enzymatic methods and subsequently analyzed.

Degree of Esterification (%DE):

DE is an important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the methyl ester. Depending upon method of analysis, %DE can be expressed as DE is measured through various techniques, but titrimetry is a longstanding, classical method of DE determination. Acetyl content of the pectin can lead to overestimation of DE content. %DE can be calculated

Degree of Methylation (%DM)

%DM is an important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the methyl ester in ratio to all esterified groups. Methoxyl content can be determined through enzymatic or alkaline demethylation. Analysis of methanol is achieved through various techniques. Most notable of these is a spectrophotometric technique HPLC method provides simultaneous measure of methyl and acetyl content.

Degree of Amidation (%DA)

%DA is another important molecular index for pectin classification that describes the extent to which carboxyl groups in pectin molecules exist as the amidated ester in ratio to all esterified groups. This measure can be a fingerprint for pectin characterization.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1Pumpkin peels:

Pumpkin was obtained directly from local central market for fruits and vegetables Khartoum North, then was peeled manually using knife, dried at 70;49˚C for 24h in oven and ground and kept for further analysis.

3.1.2 Chemicals:

All chemicals and reagent, used in this study, were of analytical grade. Nitric acid , Hydrochloric acid, Sulphuric acid, and Boric acid are from SDFCL Sd fine chem. Limited (Mumbai INDIA), Sodium hydroxide, Citric acid, Methyl red, phenol red, Tablet of catalyst are from LOBACH EMIE PVT. LTD. (Mumbai 400005 INDIA), Petroleum ether from Alpha Chemical (India), Magnesium sulphate from LANDCECH chemical (India), Sodium chloride from SCHARLAAU SPAIN and Ethanol from Drummer and sons. Co 250 (Sham Industrial city Syria).

3.1.3 Instruments:

Soxhlet, Protein unit, ovens, pH meter, and Centrifuge are from J.P Selecta, (SPIN),Water bath from DAIHAM Scientific, Co., Ltd, CORIA. ,Thermometer (OMSONS. India).

3.2 Methodology:

3.2.1 Analysis chemical composition of pumpkin peels:

3.2.1.1 Moisture content :

The moisture content was determined according to the A .O.A.C. method (1999). Samples (5 grams) were accurately weighed and dried to constant weight in vacuum oven at 70˚C and 450mm Hg for three hours. The analysis was carried out in triplicate.

Moisture % = = W_1-W_2/ S

Whereas:

W_1=weight before drying.

W_2= weight after drying.

S=weight of sample.

3.2.1.2 Ash content:

Ash was determined according to the method of A.O.A.C (1999). Samples (5 grams) were accurately weighed into relatively broad crucibles that have been reignited, cooled in desiccators and weighed. The crucible and its content were ignited in a muffle furnace at 550 ºC until light grey ash of constant weight was obtained. The analysis was carried out in triplicate samples.

. The ash content was calculated as follows:

Ash % = W_1-W_2 / W_1-W_0

Whereas:

W_0= weight of empty crucible.

W_1= weight before ashing

W_2 =weight after ash

3.2.1.3 Protein content :

Nitrogen content was determined by the semi micro kjeldahl distillation according to the method of A.O.A.C (1999). Exactly 0.2 grams of the sample were digested in a small digestion flask using half tablet of catalyst (each tablet contains I gram anhydrous sodium sulphate and0.1 gram copper sulphate), concentrated sulphuric acid (3.5 ml) were added the digest was diluted and transferred to the ammonia distillation apparatus using the minimum volume of distilled water and made alkaline with 20ml of 40% sodium hydroxide. The ammonia was distilled into 2% boric acid solution (10 ml), plus methyl red indicator (3-4 drops) for5 -10 minutes. After lowering the receiving flask clear of the condenser, the apparatus was steam distilled for further 5 minutes. The distillate was then titrated with 0.02N hydrochloric acid.

$$\mathbf{N\%}= \frac{(\mathbf{m1HCL-m1blank}) \times \mathbf{normality\ of\ HCL} \times \mathbf{14} \times \mathbf{100}}{\mathbf{sample}\ (\mathbf{mg})}$$

Crude protein = % N×6.25

6.25= refer to formula of protin

3.2.1.4 Fats content:

It was estimated according to the method of A.O.A.C (1999). Triplicate samples (2 grams) were weighed into a thimble plugged with a piece of cotton wool. The thimble was then put into a soxhlet extractor. A dray and accurately weight soxhlet flask was fitted to the extractor. Petroleum spirit of boiling range 60-80 ºC was poured to two thirds of the flask. The apparatus was then assembled and fitted to the condenser. Water was allowed to flow through the condenser and an electric heater was put on for reflux. The extraction was continued for eight hours. Then the apparatus was carefully dismantled and the liquid in the flask was evaporated to dryness in an oven at 100 ºC to a constant weight.

$$\textbf{Fat\%}= \frac{\textbf{the weight of ether extract} \times 100}{\textbf{Weight of sample}}$$

3.2.1.5 Fiber content:

It was estimated according to the method of A.O.A.C (1999). the sample from the ether extract were air dried and transferred to a dry conical flask. Then 200 ml of 1.25% sulpharic acid brought to boiling within one minute were added and the mixture then boiled gently for 30 minutes, maintaining constant volume and shaking the flask every few minutes. The residue was then filtered through apices of cotton fitted to Buchner funnel. The filtration of the solution was completed within 10 minutes .The insoluble matter was washed with hot water until the washing were free from acid ,then washed back in to original flask by mean of a washing bottle containing 1.25% sodium hydroxide (200 ml) measured at room temperature and brought to boiling .It was allowed to boil for 30 minutes and then filtered immediately through an ash less filter paper then washed with 1% HCL followed by boiling water until it was free from acid .The residue was transferred to a dried weight crucible and dried at100 ºC to constant weight .The residue was finally ashed at 600 ºC in a muffle furnace. Weight of the ash was subtracted from the weight of the dish plus insoluble residue before ashing and the difference expressed as crude fiber percentage.

3.2.1.6 Carbohydrate content:

It was estimated according to the method of A.O.A.C (1999).

Carbohydrate % = 100 – (moisture +ash+ protein+ fat and fiber %).

3.2.1.7 Calcium content:

Calcium was determined according to method described by Elmer (1993). One gram of the material was put in porcelain crucible, placed in a cool muffle furnace and ashed at 500 ºC overnight .The crucible content were dissolved into 5 ml of 25% HCL. The solution was wormed to dissolve the residue, and filtered through an acid washed filter paper in to 50 ml volumetric flask. The filter paper was washed; the solution was diluted with distillated water to volum and mixed well .using one milliliter of this solution, the amount of calcium was spectrophotometically determined at wave length of 422.7 nm using Atomic Absorption spectrophotometer (AA-6800), Japan.

3.2.2 Pectin extraction method:

3.2.2.1 Soxhlet acid extraction:

The extraction used is the referenced method (Malviya, 2010). It can be carried out in two steps.

3.2.2.1.1 Pectin extraction :

Pectin was extracted under reflex in condensation system using water acidified for 1hr with acid to pH 2. Temperature of extraction media was maintained at 80 ºC, and extraction time was adjusted to about 6 h. The extractor thimble was a Whatman cellulose thimble with 33 internal diameter and 80 mm external length. Dried powdered pumpkin peel was taken in soxhlet and reflex was continued for 6 h.

3.2.2.1.2 Pectin precipitation :

Hot acid extract was pressed in cheese cloth bag and the cake was cooled to 4 ºC. Pectin precipitated by alcohol treatment 2:1 (v/v) followed by continuous stirring for 15 min and allowed to stand for 2 h for better precipitation. This allow to filter pectic substances because pectin remains float on surface of alcohol. Floating pectin coagulate was filtered through cheese cloth, washed with alcohol 70 % and pressed. Pressed pectin was further dried to constant weight at 35-45 ºC in hot oven. Hard pectin cake was ground and sieved through 20 mesh sieve size, stored in desicator for further use.

3.2.2.2 Acid extraction method:

The acid extraction used is according to Crandall et al., (1978[a]) method with slight modifications. Four hundred ml of distilled water (DW) was measured into a 2000 ml Erlenmeyer flask and maintained at the desired temperature 80 ºC using the stirring hot plate or the shaking water bath which are weighed 100 g peel was added to the water. Measured amounts of acid using different acids (Nitric and Citric Acids) were added to the peel-water mixture until the desired pH 2. The mixture was agitated at a constant temperature until the desired extraction time had elapsed. The pH and temperature were recorded and the mixture was allowed to cool in an ice water bath until it reached 55 ºC. The mixture was centrifuged at 5050 rpm for 10 min. The filtrate was vacuum filtered using filter paper and the solids were resuspended in 400 ml of 60 ºC DW water for 5 min. Acid extracted pectin was kepet after overnight precipitation. The pectin was separated from the alcohol solution using a double layer of cheese cloth and the samples were washed three times with 70% diluted alcohol to remove any impurities. The resulting pectin was dried under vacuum at 50 ºC in aluminum sample dishes until all moisture was removed. Pectin was cooled, weighed and ground using a mortar and pestle. Ground pectin was stored in small plastic sample bags. Both of the two extraction procedures were done twwice by using nitric and citric acids. The amount of pectin was calculated according to the following equation:

$$\textbf{Pectin yield} = \frac{\textbf{Weight of extracted pectin} \times \textbf{100}}{\textbf{Weight of dried peel}}$$

3.2.3 Effect of time in pectin extraction:

The effect of time on pectin yield was examined to determine if increasing extraction time would increase pectin yield. The extraction time was set at 30, 60 and 90 min; according to the extraction time was used in the referenced method Crandall et al., (1978[a]), with slight modification. Effect of time on acid extracted pectin yield was done at extraction conditions of 80 ºC, pH 2 using 1 N nitric acid, and solid to liquid ratio of 1:4 g/mL using water bath.

3.2.4 Chemical analysis of extracted pectin:

3.2.4.1 Moisture content :

The moisture content of pectin was determined according to the method of A.O.A.C (1995). Triplicate samples (1 gram) were weighed in dried and weight aluminum dishes. Samples were then dried for 4 hours at 105 ºC (20 mmHg). They were then cooled in desiccators and weight to constant weight. The moisture content was calculated as follows:

Moisture % = = W_1-W_2/ S

Whereas:

W_1=weight before drying.

W_2= weight after drying.

S=weight of sample.

3.2.4.2 Ash content:

The ash content of pectin was determined according to the method of A.O.A.C (1995). were used for analysis. Triplicate samples (1 gram) were weighed into previously ignited, cooled and weight crucibles. Sample then was ignited at 600 ºC for 3 hours, cooled and weight to constant weight. The ash content was calculated as follows:

Ash % = W_1-W_2 / W_1-W_0

Whereas:

W_0= weight of empty crucible.

W_1= weight before ashing

W_2 =weight after ash

3.2.4.3 Ash alkalinity:

The ash alkalinity of pectin was determined according to (Owens et al., 1952). The ash prepared was dissolved in 25 ml of 0.1 N HCL, heated gently and then titrated with 0.1 N sodium hydroxide using phenolphthalein indicator. The alkaline number of an ash was calculated as the number of milliters of acid required to neutralize one gram of ash.

3.2.4.4 Equivalent Weight:

Equivalent weight was determined by Ranganna's method (1995). Triplicate samples (0.5gm) were weighted in to 250 ml conical flask and moistened with 5 ml ethanol. The product was mixed with 1 gm sodium chloride and 100 ml of distilled water. The mixture was stirred vigorously and free acidity was determined by direct titration against 0.1 N sodium hydroxide using phenol red as an indicator. Also blank containing the same quantities of reagents was tested. This titre is known as initial titre (IT) or free acid titre. The equivalent weight was calculated as follows:

Equivalent weight = weight of sample (mg) / Meg of sodium hydroxide

Meq (Miliequivalent of sodium hydroxide) = Normality × Titre volume of Noah

3.2.4.5 Methoxyl Content:

Determination of MeO was done by using the Ranganna's method (1995). The neutral solution was collected from determination of equivalent weight and 25 ml of sodium hydroxide (0.25 N) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min. After 30 min 25 ml of hydrochloric acid (0.25 N) was added and titrated against 0.1 N NaOH.

Methoxyl content was calculated by following formula:

$$\textbf{Methoxyl content \%} = \frac{\textbf{Meq of NaOH } \mathbf{3.1 \times 100}}{\textbf{Weight of sample}}$$

Whereas:

Meq of NaOH = normality of NaOH titre figer

3.1 refer to formula weight of methoxyl group.

3.2.4.6 Degree of esterification (DE):

The DE of pectin was measured on the basis methoxyl content (Owens et al., 1952)

From IT and ST obtain the degree of esterification and Anhydrouronic acid (AUA) content was calculated as follows:

Degree of estertification (DE) = ST × 100/ ST+ Corrected IT

The IT corrected for the ash alkalinity

3.2.4.6 Acetyl content:

Acetyl content was determined according to Owens et al., 1952). Triplicate samples (0.5 gm) each pectic substance was weighed in to flask and 250ml of 0.1N NaOH were added. The flask was covered, shaken and left overnight. The solution was then diluted to 50 ml from which 20 ml were taken and placed in distillation apparatus .Twenty milliliter magnesium sulphate sulphuric acid solution (100 g magnesium sulphate and 1.5 gm sulphuric acid diluted to180 ml) were added. The solution was then steam distillated and 10 ml of distillate were collected. The acetic acid of the distillated was then titrated with sodium hydroxide (0.05 N) to phenol red point. The titre was corrected for the reagents blank. The acetyl content (formula weight 43) of sample was calculated according to the following equation:

$$\textbf{Acetyl content (\%w/w)} = \frac{(\textbf{ml NaOH})\ (\textbf{normality of NaOH}) \times \textbf{43}}{\textbf{Weight of sample}\ (\textbf{gm})\ \textbf{in the liquot}}$$

Whereas:

MI NaOH = volume of required to titre distillate –volum of NaOH required to titre distillate blank run.

43=formula weight of Acetyle.

3.2.5 Statistical Analysis

The data of this study was analyzed using computer statistical page of social science soft ware excel. Data was expressed as mean ± standard deviation (STD). The statistical significance was considered at $P < 0.05$. All the experiments were organized using a completely randomized design with three replicates, repeated for reproducibility. The data obtained from the measurements were subjected to T-test was used to compare different between properties of pectin extracted by different acid. All measurements were carried out in triplicate for each sample. The experimental data were reported as the means ± SD of independent trials (Wagner, 1985).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chemical compositions of pumpkin peel:

Chemical compositions were presented in Table 4.1 expressed on dry basis. The moisture content was 20.1%. The Ash content of pumpkin peel was 7.1 %. The protein content of pumpkin peel was 3.2%. The fiber content was 10.15 %. The fat content was 2.3. The carbohydrates of pumpkin peel was 57.15 % and calcium was 0.308 %. This result was disagreed with Ibrahim (2008) who found that the chemical compositions of pumpkin peel on weight basis as fallow. The moisture content was 84.7- 90.26%. The Ash content of pumpkin peel was 0.81-1.35%. The protein content of pumpkin peel was 1.3-1.64%. The fiber content was 3.21-4.82%. The fat, carbohydrates and contents calcium were 0.04, 5.17 and 0.062 % respectively.

Table 4.1: Chemical compositions of pumpkin peels on dray basis

Parameter	**%**
Moisture	20.1
Ash	7.1
Protein	3.2
Crud fiber	10.15
Fats	2.3
Carbohydrate	57.15
Calcium	0.308

Values are means of 3 replicates for each parameter

4.2: Effect of different method on pectin yield:

Optimal extraction conditions of this study use a nitric and citric acid to adjust pH to 2 extracted for 60min at 80ºC, Soxhlet extraction for six hours alcohol precipitation two hours but in acid extraction without soxhlet, alcohol perception was ten hours. The results obtained were (7.72 % nitric acid and 6.80 % citric acid) using soxhlet acid extraction method and (6.24% nitric acid and 5.36% citric acid) acid extraction method without using soxhlet. As shown in Figure 4.1, the result indicated that using soxhlet acid extraction method higher yield than acid extraction method was obtained without using soxhlet to extract pectin from pumpkin peel. The result obtained in this study disagreed with Desalination (2008) who reported that, for microwave extraction, the greatest total amount of pectin yield was found to be 5.27% on a dry basis for 15 minutes of extraction, although the greatest amount of material per unit time (%/min) was obtained after 5 minutes. This amount was the same as that extracted by using Soxhlet extraction for three hours from orange peel.

4.3: Effect of different acids on pectin yield:

The extracted pectin from pumpkin peels in this study was 6.24% by using nitric acid and 5.36% by using citric acid .The study showed that nitric acid produced higher than citric acid respectively as shown in figure 4.1. Significant difference p0.05 was observed between two acids. The result was higher compared with the range of 0.253-0.233 % for pectin extraction from alcohol insoluble solids from pumpkin peel were reported as the use of hydrochloric acid is to adjust pH to 2 extracted for 60min at 80ºC (Ibrahim, 2008). It disagree with Sayah *et al,* (2014),who extracted pectin from a steam distillated and non conditioned orange peels using different acids .

Citric acid gave the best average yield (25.71% ± 0.007) sulfuric, hydrochloric and acetic acids gave a very low average yield of pectin, the average yields obtained are respectively 6.49% ±0.005, 7.96% ± 0.005, and 10.19% ± 0.006 .

4.4: Effect of extraction time in pectin yield:

The effect of extraction time on pectin yield was examined to determine increasing extraction time would increase pectin yield. The results were shown in Figure 4.2. There was increasing in pectin yield between 30and 60min of extraction times. Since there was no noticeable trend occurred with increasing extraction time from 60 min to 90 min, so, the suitable extraction time was 60 min. The result of this study disagreed with (Campbella, 2006). There was increasing in pectin yield between 0-45min .No noticeable on time trend occurred with increasing extraction time from 45 min to 90 fallowing min

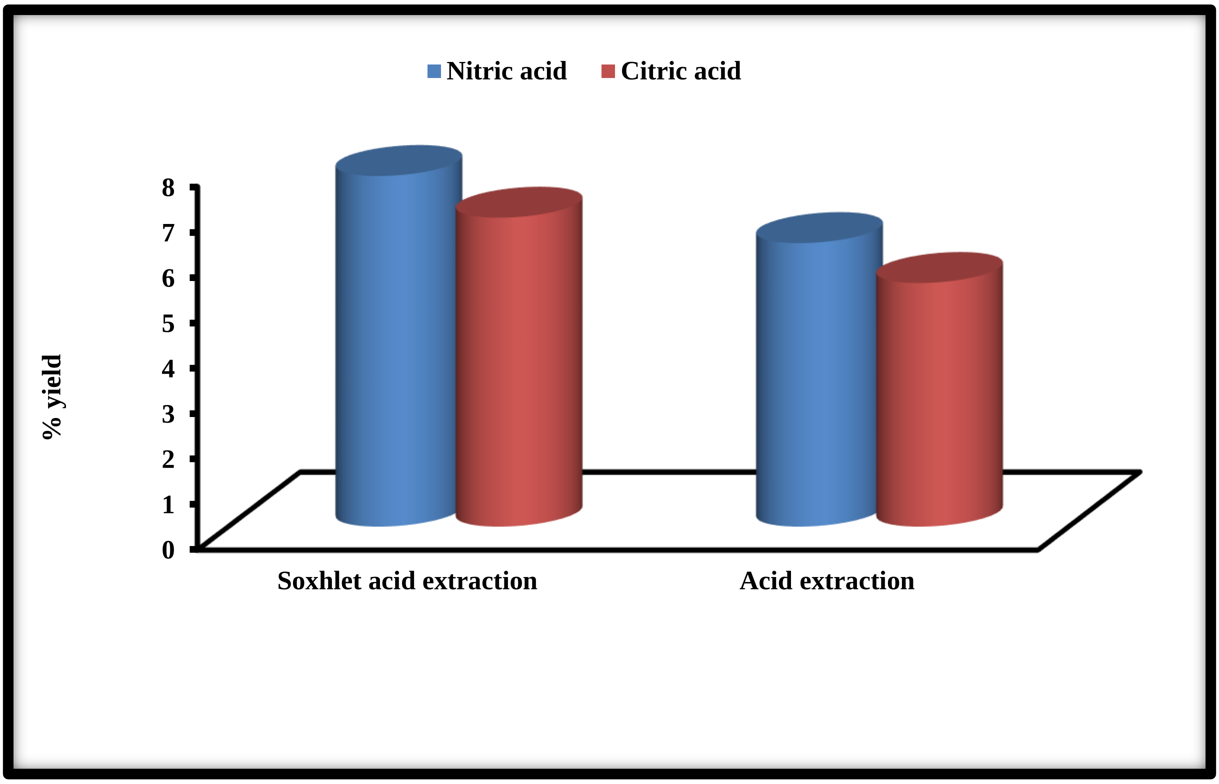

Fig 4. 1: Pectin yield by using different methods and acid

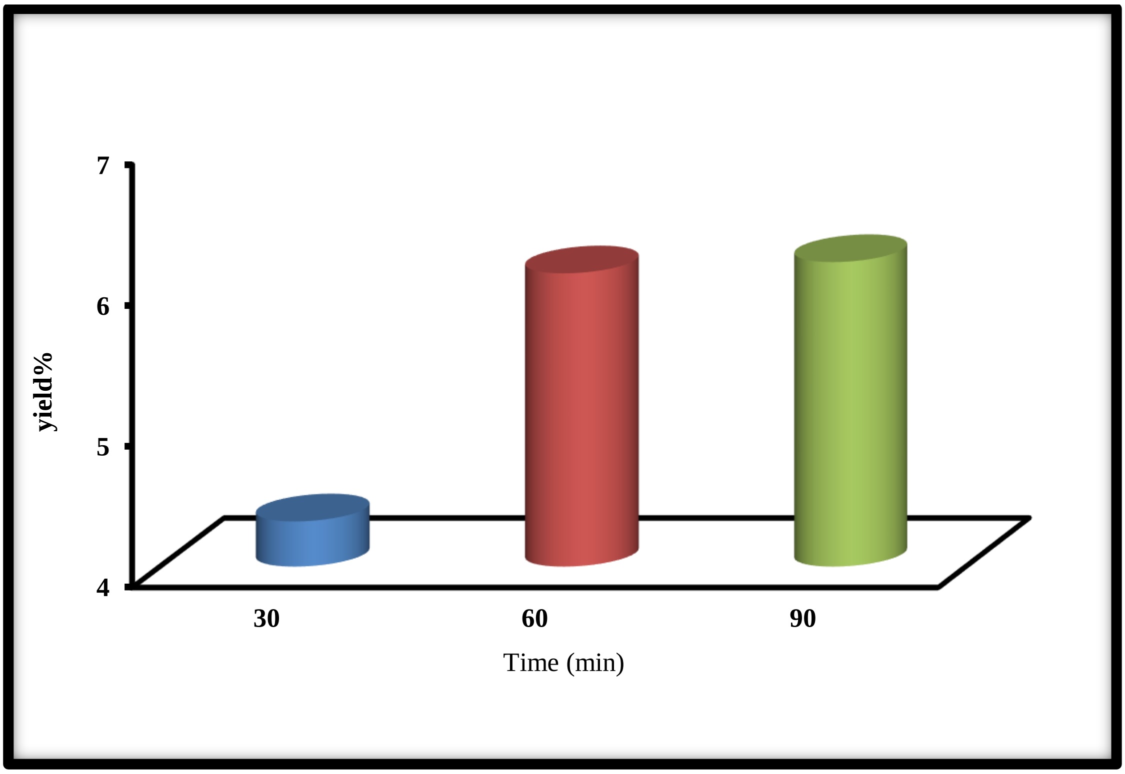

Fig 4. 2: Effect of extraction time on pectin yield

4.5 Chemical composition in pectin pumpkin peels:

The results for chemical composition of pumpkin peels pectin were discussed as follows:

4.5.1 Moisture content:

Moisture content of pectin pumpkin peels extracted by nitric and citric acids was 5.54±0.01 % and 5.42±0.0 % respectively as shown in figure4.3. Significant difference p0.05 was observed between two samples. The result is lower if compared with the value the ranging from 7.88 - 8.96% for grapefruit peels pectin Mohamed (1999). The values obtained also higher than the range of 5.03 - 5.04% for mango pulp pectin reported by Abderahman (2002).

4.5.2 Ash content:

Ash content of pumpkin peels pectin extracted with nitric and citric acids was 3.17±0.006 and 2.96 ±0.006% respectively as shown in figure 4.4 There was a significant difference p0.05 between the two samples. The result is higher if compared with the range of 1.56-1.65% for (lemon) 0.49-, 0.81-4.83% (orange) and 0.49-8.05% (apple) by Abderahman (2002) Mohamed (1999) reported arrange of 1.8% to 2.0% for grapefruit peels pectin.

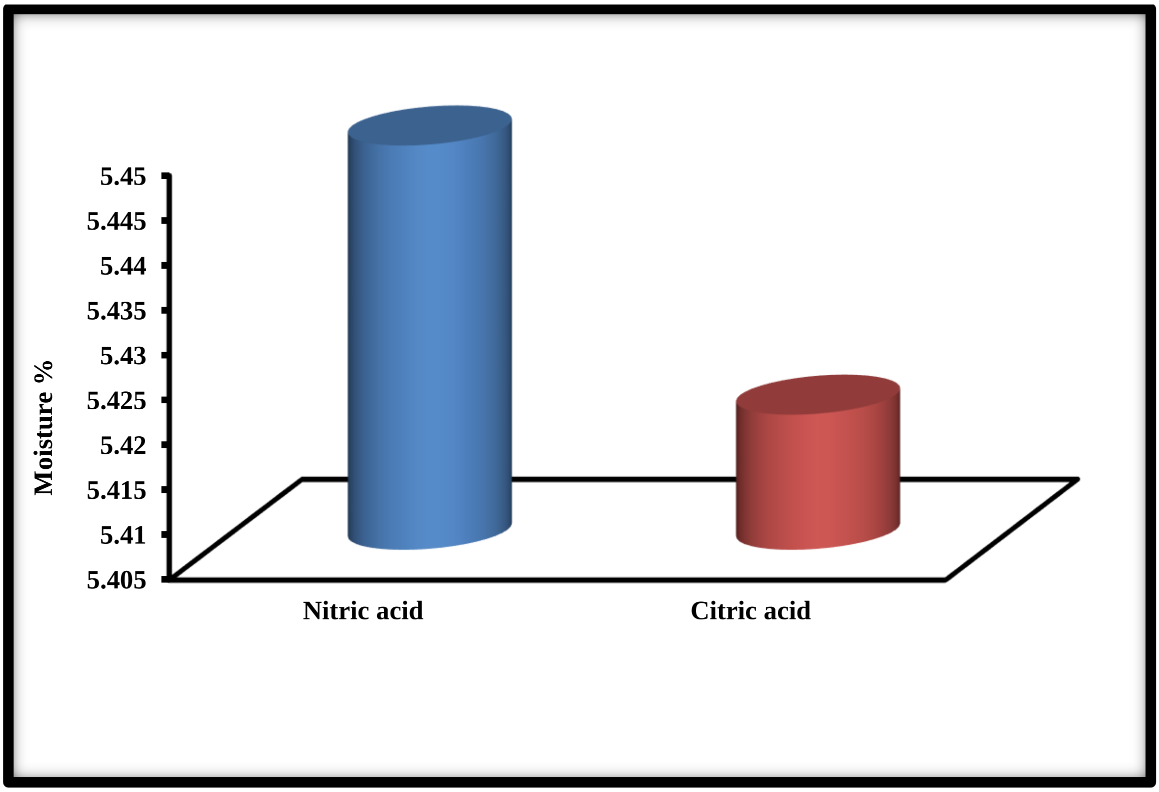

Fig 4. 3: Moisture content of pectin of pumpkin peel

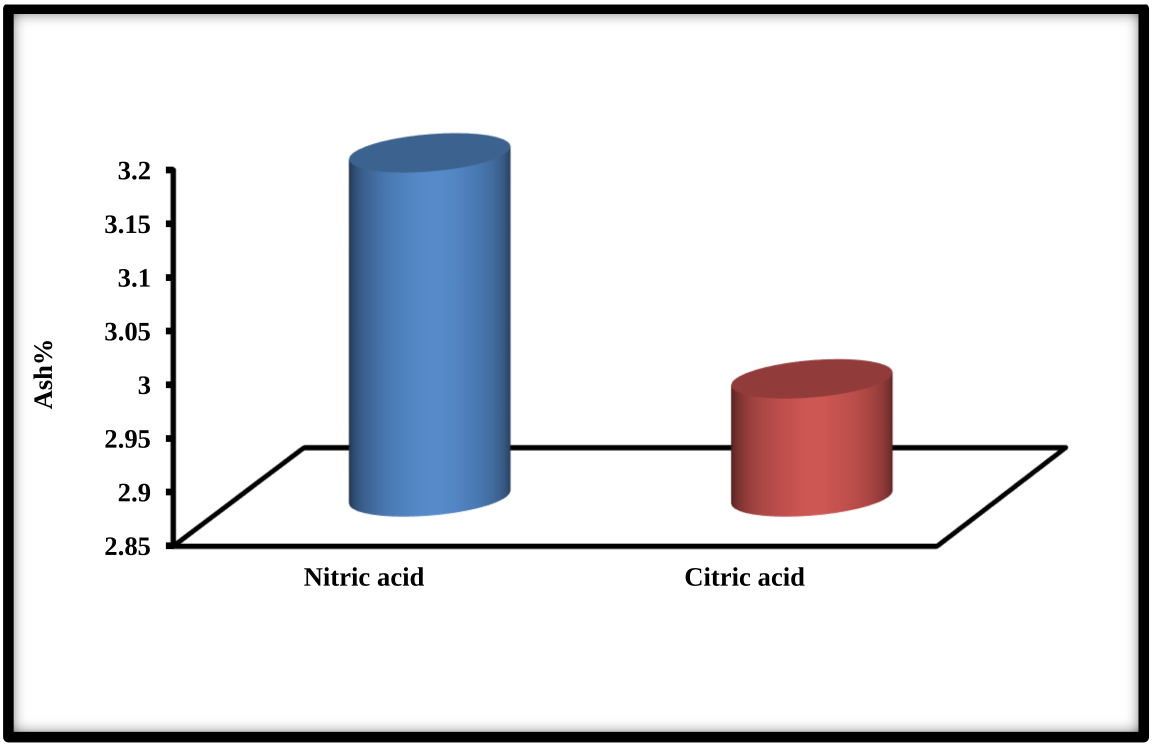

Fig 4. 3: Ash content of pectin of pumpkin peel

4.5.3 The equivalent weight:

The equivalent weight of extracted pectin from pumpkin peels by using nitric and citric acids was 1250 ± 0.0 and 1250 ± 0.0 g/mol respectively as shown in figure 4.3. The results showed that no significant difference p0.05 between two sample but higher than cocoa husk pectin (510.68 - 645.19 g/mol) that reported by Ramli and Asmawati (2011) and the ones reported by Mohamed (1999) for grapefruit peels pectin for citrus pectin (620-749 g/mol). The equivalent weights obtained in this study was lower than range of 1389-2003 g/mol for mango pulp reported by Abderahman (2002) and than apple pomace pectin (833.33 to 1666.30 (Kumar & Chauhan, 2010) and (263,000–303,000 g/mol) for ambarella peels reported by Koubla (2008).

4.5.4 Methoxyl content:

The methoxy1 content of pumpkin peels pectin extracted by nitric and citric acids were 6.20±0.10% and 7.23±0.89% respectively as shown in figure4.6. No significant difference p0.05 was observed between two samples. The methoxyl obtained in this study was lower than those found by Ali, (2014) for lemon pomace (10.25±022%) and the one reported by Madhav and Pushpalatha, (2002) for pomelo peel (8.57%), Lime (9.92%), passion (8.81-9.61%); and it was similar to peel of mango (7.33%), banana (7.03%) (Madhav and Pushpalatha, 2002) but higher than dragon fruit pectin (2.98% to 4.34%) (Ismail *et al*, 2012). Methoxyl content is an important factor in controlling the setting time of pectin and the ability of the pectin to form gels (Constenla and Lozano, 2003).

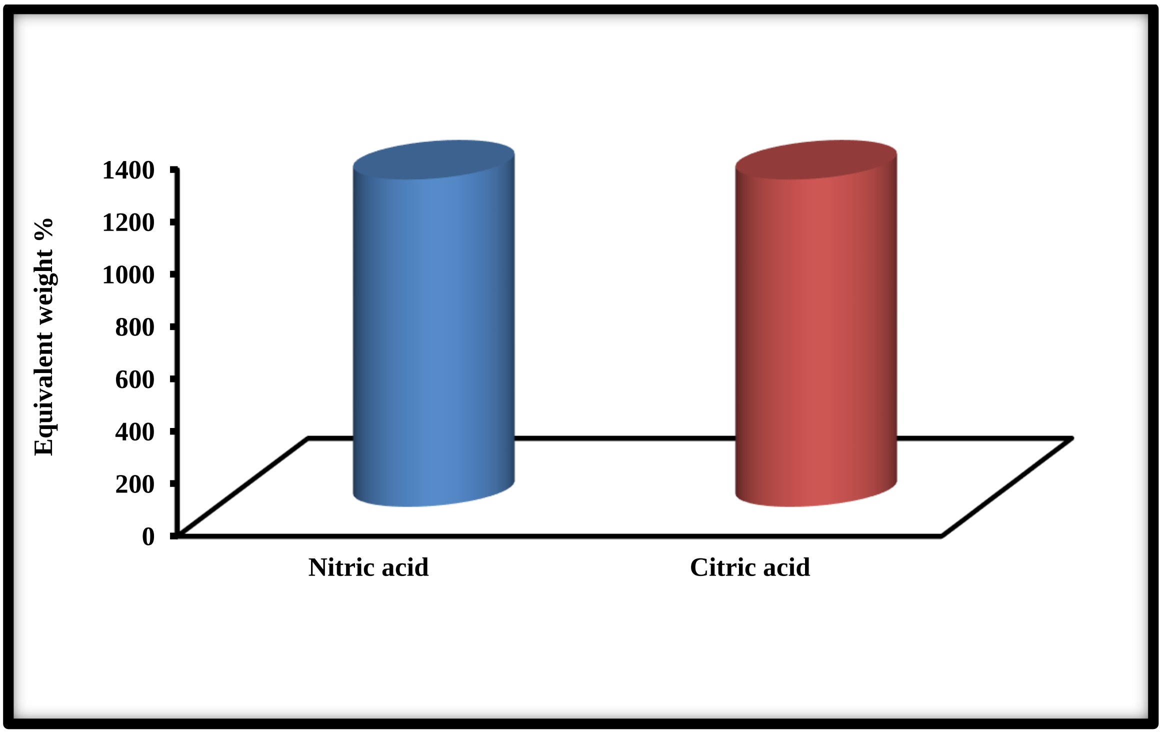

Fig 4. 4: Equivalent weight of pectin of pumpkin peel

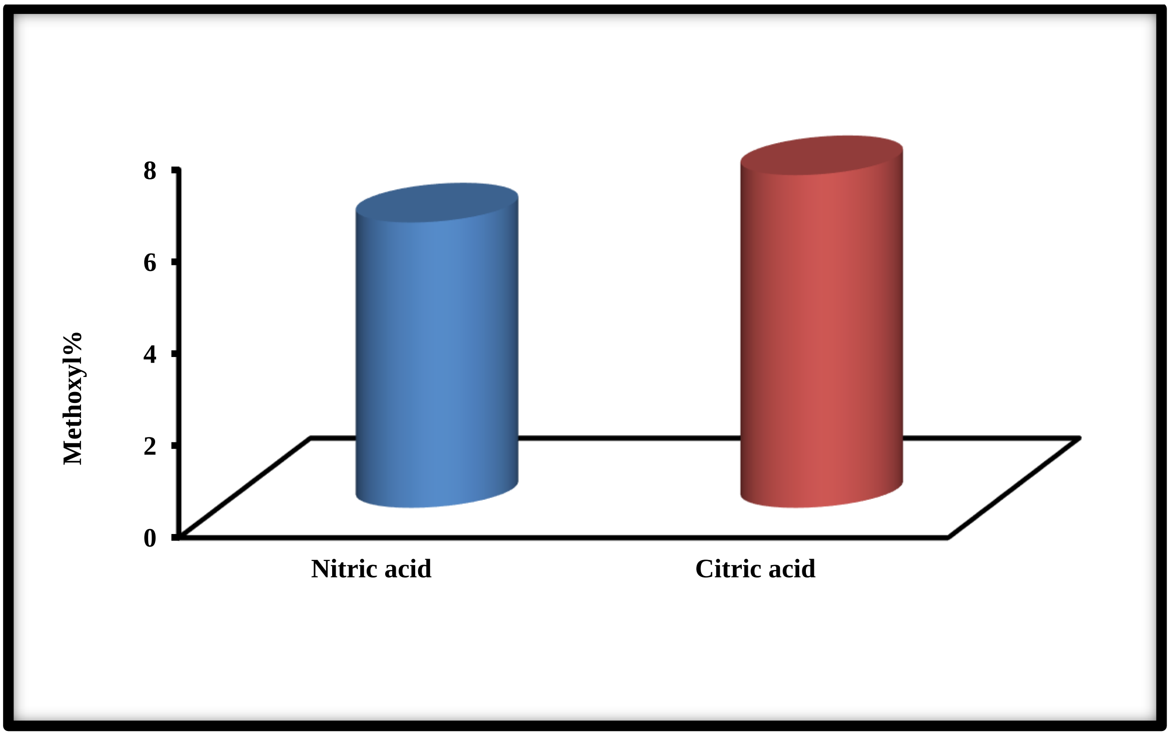

Fig 4. 5: Methoxyl content of pectin of pumpkin peel

4.5.5 Acety1 content:

Acety1 content of pumpkin peels pectin extracted with nitric and citric acids was 0.43±0.01 and 0.43±0.03 % respectively. There was no difference p0.05 between two samples. The result agreed with the finding of Mohamed (1999) who reported 0.46-1.63% for grapefruit peels pectin and it was higher than the results reported by Abderahman (2002) for acety1 content in mango pulp pectin (0.117% to 0.314%). The acety1 group in pectin substances have significant role on account of their effect on the jelly formatting ability (Abderahman 2002). Also the finding of this study was lower than 4-6% the one reported amarelle peels as reported by Koubla et al (2008).

4.5.6 Degree of esterification:

The results in (Figure 4.8) for the degree of esterification of pumpkin peels pectin extracted by nitric and citric acids were 66.53±0.058 and 66.57±0.058 % respectively . .There was no difference p0.05 between two samples. The value agreed with the values reported by El-tiny et al., (1982) for mango peels pectin (66.9%) but higher than that obtained by Mohamed (1999) for grapefruit peels pectin, who reported 51.01 - 51.24% and by Koubla et al (2008) for ambarella peels (50–58%).

The values of degree in esterification of this study are lower than the those reported by Eltinay et al (1982) Abderahman (2002) and Ali (2014) for pumpkin pectin (73.9 - 86.8%), for mango pulp pectin(87.0%) and for lemon pomace pectin (79.51±0.36 and 70.39±4.20%) respectively.

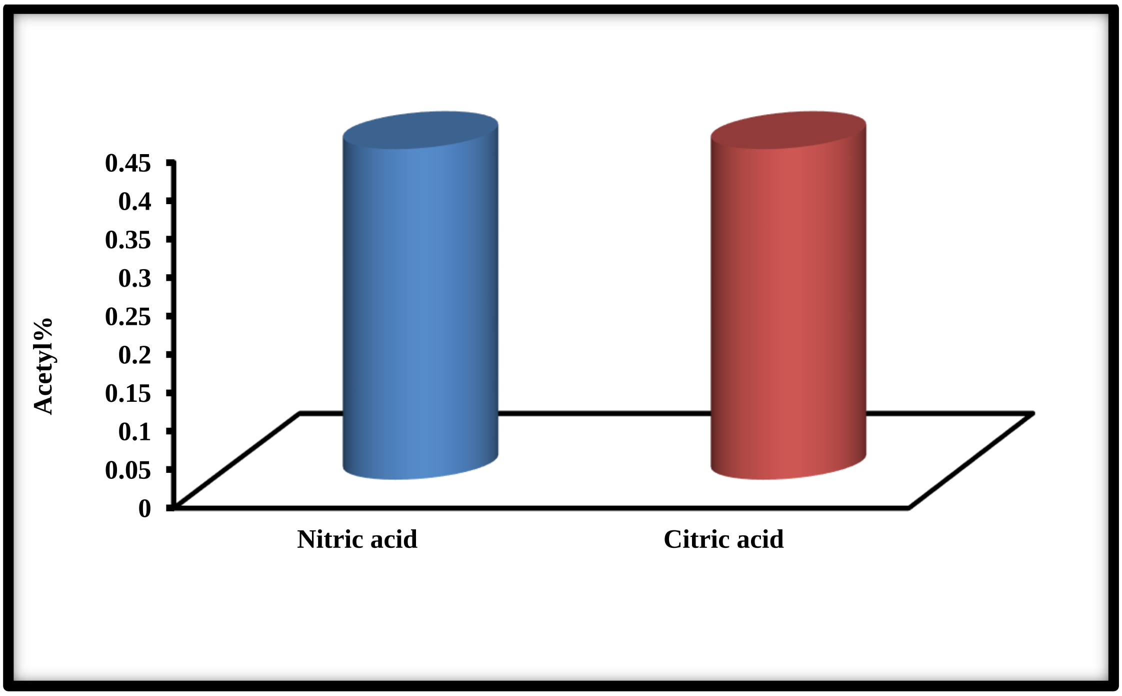

Fig4. 6: Acetyl content of pectin of pumpkin peel

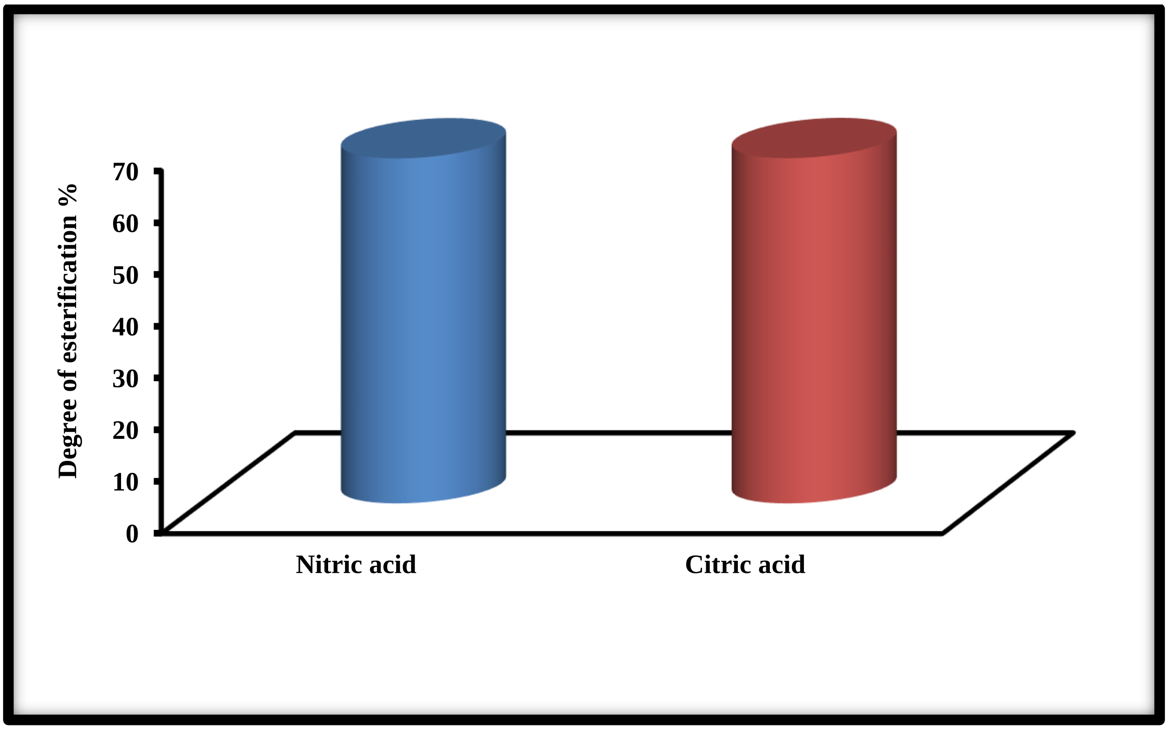

Fig 4. 7: Degree of esterification of pectin of pumpkin peel

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion:

Pumpkin peel has high level and good properties of pectin, using soxhlet and nitric acid at 60min for extraction time give the highest yield.

5.2 Recommendations:

1. Techno-economic study on production of pectin from pumpkin peel.

2. Average the commercial production of pectin.

3. Further work is needed to achieve application.

REFERENCES

AOAC., 1995. Official Methods of Analysis of the Association of Official Analytical Chemistry. 16th Edn., AOAC International, Washington, USA., Pages: 1141.

A.O.A.C. (1999). Association of official Analytical chemists . 16th Edition, Association of Official Analytical Chemists, Maryland.

Abdel Rahman , N.A.(2002). Characterization of pectic substance of Abu- samaka cultivar in relation to their influence on mango pulp concentrates M.Sc., thesis. University of Khartoum. Sudan .

Ali,S. M, Md. Jiaur M. (2014). Isolation and Characterization of Pectin Extracted from Lemon Pomace during Ripening. Journal of Food and Nutrition Sciences. Vol. 2, No. 2, , pp. 30-35

Aravantinos-Zafiris, G. V. Oreopoulou. 1991. The effect of nitric acid extraction variables on orange pectin. Journal of Science in Food and Agriculture. 60: 127-9.

Barbara, S. and Murkovic, M. (2004). Changes in Chemical Composition of Pumpkin Seeds During the Roasting Process.

Bates, R.P.; Morris, J.R. and Grandall, P.G.(2001). Principles and practices small and medium scales fruit juice processing. FAO Agric. Services Bulletin 146.

Braddock, R.J. 1999. Handbook of citrus by-products and processing technology. John Wiley & Sons, Inc. New York. 191-7.

Benjamin, O., Silcock, P., Leus, M., Everett, D. W. (2012). Multilayer emulsions delivery systems for controlled release of volatile compounds using pH and salt triggers. *Food Hydrocolloids, 27/1,* 109–118.

Bottger, I. (1990). Pectin application—Some practical problems. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.), Gums and stabilisers for the food industry. Oxford, UK: IRL Press, 5, 247–256.

Braddock, R.J. 1999. Handbook of citrus by-products and processing technology. John Wiley & Sons, Inc. New York. 191-7.

Campbell, (2006) Extraction of pectin from watermelon rind, Bachelor of Science in Biosystems Engineering Oklahoma State University Stillwater, Oklahoma

Carbonell, M.E.; Wessel, B.L.; Verela, F.; and luciano, B.(1990). Pumpkin (cucurbita moschata) Breeding priorities Based on a survey of Puerto Ricon consumers. J. Agric Univ. Puerto Rico74:229-236.

Constenla D, Lozano JE. (2003). Kinetic model of pectin Demethylation Latin American Applied Research,33, 91-96.

Crandall, P.G., R.J. Braddock, A.H. Rouse. (1978^a). Determining the yield and quality of pectin from fresh peel and pectin pomace. Procedures of the Florida State Horticulture Society. 91: 109-11.

Desalination, (2008) Comparison between different technic for water based extraction of pectin from orange peel. 5January vol.218 (1) :229-237 .syeoh, J.shi, T. A. G. langrish.

Dinu, D.(2001), Extraction and Characterization of pectins from wheat bands Rumanian Biotechnology letera,6.37-43

Dixon, W. D. (2008)."Characterization of Commercial Pectin Preparations by Spectroscopic and Chromatographic Techniques." East Tennessee State University. Electronic Theses and Dissertations. Paper 1910. http://dc.etsu.edu/etd/1910

El Awad, O.B.(1980). Chemical composition of Sudanese. Food-Staff Vegetables and fruits. Sudan J.Fd.Sci. and Techol.12:64-71.

El shafie, A.S. (1981). Isolation, fractionation, and characterization of pumpkin pectic substance .M.Sc. thesis, University of Khartoum, Sudan.

Elmer, M.M.(1993). Characterization of Apple fruit Pectic substances. J .fd. i .Sci. and Technol. 30 (2) :17-19.

El Tinay, Aa.H.; El Shafie, A. S. and Nour, A. M. (1982). A chemical study of Pumpkin pectic substances Dep . Of Bio. Faculty of Agriculture, University of Khartoum, Sudan.

Fu, C. L.; Shi, H.; Li, Q. H. A. (2006) review on pharmacologicalactivities and utilization technologies of pumpkin.Plant Foods Hum Nutr, 61,70-77..

Hwang Jae-Kwan; Kim, Chul-Jin and Kim, Chong-Tai(1998), Extrusion of Apple Pomace Facilitates Pectin Extraction. Journal of Food science, 63, 841-844.

Ibrahim, Y. A. (2008). Isolation and Characterization of Pectic Substance of pumpkin peels . M.SC. These University of Khartoum, Sudan.

Inari, T; Yamauchi, R ;Kato, and Takeuhi , T.(2002).Changes in pectic polysaccharides During the Ripening of Cherry Tomato fruits. J. fd. Sci. Techol. Res.8:55-58.

IPPA: International Pectin Producers Association. 2001. What is pectin? Available on line at.ippa.info/commercial_production_of_pectin.htm. (Accessed September 2004).

Ismail NSM, Ramli N, Hani NM, Meon Z. (2012). Extraction and characterization of pectin from dragon fruit (Hylocereus polyrhizus) using various extraction conditions. Sains Malaysiana,41: 41-45.

Jimenez, A.; Guillen, R; Sanchez, C.; Fernandez-Bolanos, J. and Heredia, A.(1996). Molecular Weight and Ionic Characteristics of olive Cell Wall polysaccharides During Processing .J. Agric. fd.Chem.44: 913-981.

Jun, H; Lee, C.;Song, G. and Kim,Y.(2006). Characterization of the pectic polysaccharides from pumpkin peel .Faculty of Biotechnology Dep. Anatomy: Woosuk University Korea 39:554-561.

Kalapathy, U. and Proctor, A.(2001). Effect of Acid Extraction and Alcohol Precipitation Conditions on the yield and Purity of Soy hull Pectin .J. fd. Chem. 73:393-397.

Koubla, B.B. Mbome.T. I., Kansci, G, Tchouanguaeb. Mbiapo, F., Crepeau, M.J Thibault,J. F. and Kallet, M. C. (2008) phesio Chemical properates of pectin From ambarella peels (Spondias cytherea). obtain using different extraction condition.Food Chemistry 106 page 1202-1207

Kumar A, Chauhan, GS. (2010). Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor. Carbohydrate Polymer, 82: 454-459.

Madhav A, Pushpalatha PB. (2002). Characterization of pectin extracted from different fruit wastes. Journal of Tropical Agriculture,40: 53-55.

Malviya R., Srivastava P., Bansal M., Sharma P.K.(2010). Mango Peel Pectin as Super disintegrating Agents .Journal of Scientific and Industrial Research69,688-690 .

Maria Helene Canter i-Schemin, Heloisa Cristina Ramos Fertonani, Nina Waszczynskyj and Gilvan Wosiacki (2005), Extraction of Pectin From Apple Pomace. JOURNAL BRAZILIAN ARCHIVES OF BIOLOGY AND TECHNOLOGY Vol.48,n.2:pp, 259-266 ISSN 1516-8913 .

May, C. D. (1990). Industrial pectin : Sources Production and Applications Carbohydrate Polymers,12(1): 79-99.

Maynard, D. N.(1996). Performance of Tropical Pumpkin Inbreds and Hybrids. Proc. VIth EUCARPLA meets. CuCurbit Genetics and Breeding.26-29 May 1996, Malaga, 59-65.

Mohamed, H. A. (1999). Extraction, Fraction and Characterization of Pectic Substance of Grapefruit peels . M.SC.University of Khartoum, Sudan.

Murkovic, M., Mülleder, U., Neunteu, H. (2002). Carotenoid Content in Different Varieties of Pumpkins. *Journal of Food Composition and Analysis, 15,* 633.

Murkovic, M.Piironen, V.Lampi, A.: Kraushofer.T. and Gerhard, S.(2004). Changes in Chemical Composition of Pumpkin Seeds During The Rosting Process for Production of Pumpkin seed Oil (Part 1:nonvolitite compounds). Food Chem . 84:359-365.

Ngouémazong, D. E., Tengweh, F. F., Fraeye, I., Duvetter, T., Cardinaels, R., on Loey, A. V., Moldenaers, P., Hendrickx, M. (2012). Effect of de- methylesterification network development and nature of Ca^{2+}-pectin gels: Towards understanding structureefunction relations of pectin. *Food Hydrocolloids*, 26, 89-98.

Obeidalla, A. A. and Riley, J . J. (1984). Development of the Horticultural Potential of Kordofan Region of Sudan .A paper Presented in Eighth African Symposium on Horticulture Number 143.

Owens, H. S.; McCready, R. M.; Shepherd, A. D.; Schultz, T. H.; Pippen, E. L.; Swenson, H. A.; Miers, J. C.; Erlandsen, R. F. and Macaly, W. D. (1952). Methods Used at Western Regional Research -Laboratory for Extraction and Analysis of Pectin Material. AIC 340. Albany, Calif. (Cited from Abdel Rahman, 2002).

Phatak, L, K.C. Chang, G. Brown. 1988. Isolation and characterization of pectin in sugar-beet pulp. Journal of food science. 53(3): 830-3.

Phillips, G.O.(2000).Colloids: Apartnership With Nature. In K.Nishinaari(Ed.), Hydrocolloids. Part 2. Fundamentals and Applications in food Biology and Medicine (Pp 3-15). Amsterdam: Elsevier.

Pornsak Sriamornsak. (2003). "Chemistry of Pectin and its Pharmaceutical Uses: A Review ". Silpakorn University International Journal 3(1-2):206.

Ptichkina, N.M.(2000).Potential in the Lower Volga Region. Khraneniye Pererabotka Selskohozjaistvennogo Syrja,11:42-45.

Ptichkina, N.M.; Markina, O.A. and Rumyantseva, G.N.(2008). Pectic Extraction from Pumpkin with the aid of Microbial Enzymes.J. Food Hydrocolloids.22:192-195.

Ptichkina, N.M.; Novokreschonova, L.V.; Piskunova , G.V. and Morris. E.R.(1998). Large Enhancements in Loaf Volume and Organoleptic Acceptability of wheat bread by small Additions of pumpkin powder : possible Role of Acetylated pectin in stabilizing Gas-cell structure. J. Food Hydrocolloids,12:333-337.

Ralet, M-C.; Bonnin, E. and Thibault, J . F . (2005) Pectins. In A. Steinbiichel and S.K.Rhee (Eds., Polysaccharides and Polyamides in the Food Industry (Pp.351-386).Wiley-VCH Verlag.

Ramli N, Asmawati (2011). Effect of ammonium oxalate and acetic acid at several extraction time and pH on some physicochemical properties of pectin from cocoa husk(Theobroma cacao). African journal of Food Science,5: 790-798.

Ranganna S. (1995). Hand book of analysis and quality control for fruits and vegetable products (2ndEd.). New Delhi: McGraw Hill publishing Co. Ltd. pp. 33-43.

Rolin, C.(2002). Commercial Pectin Preparation. In G.B. Seymour and J.P. Knox (Eds.), Pectins and their Manipulation (pp.222-241). Oxford: Black Well Publishing, CRC Press.

Sayah, et..al (2014). Comparative Study on Pectin Yield According To the State of the Orange Peels and Acids Used. International Journal of Innovative Research in Science, Engineering and Technology Vol. 3, Issue 8, August 2014

Schols, H. A., DAss, P.J.H., BAkX, E. J. and VORAGEN, A. J. (1998) (structural Features of Natire and Commercially Extracted pectin, Gums and stabilisers for the food Jndustry; Wrexham, The Royal Society of chemistry.

Shkodina, O.G.; Zeltser, O.A.; Selivanov, N. y. and Ignatov, v. v. (1998).Enzymic Extraction of pectin Preparations from Pumpkin. Food Hydrocolloids, 12:313-316.

.

Simpson, B.K., K.B. Egyankor, A.M. Martin. 1984. Extraction, purification, and determination of pectin in tropical fruits. Journal of Food Processing and Preservation. 8(2): 63-72.

Souza, J. R. R., Carvalho, J. I. X., Trevisan, M. T. S., Paula, R. C. M., Ricardo, N. M. P. S., Feitosa, J. P. A. (2009). Chitosan-coated pectin beads: characterization and in vitro release of mangiferin. *Food Hydrocolloids, 23/8*, 2278-2286.

Trease, G. E. and Evans, W. C. (1989). Pharmacosy. 13th edition. Bailliere Tindall, London(Pp 46:203,542-649.

Van Buren, J.P. 1991. Function of pectin in plant tissue structure and firmness. In The chemistry and technology of pectin. Academic Press. California.

Vincken, J. P., Schols, H. A., Oomen, R. J. F. J., Beldman, G., Visser, R. G. F. and Voragen, A. G. J. (2003). Pectin – the hairy thing. In: Voragen, A. G. J., Schols, H. and Visser, R., Editors, Advances in Pectin and Pectinase Research, Kluwer Academic Publishers, Boston, Dordrecht, 47–59.

Venkey, (2009) Properties of Pectin. Classle learning social Tue, 06/02/2009 - 10:11 https://www.classle.net/book/properties-pectin

Voragen, A. G. J.; Pilnik, W.; Thibault, J. F.; Axelos, M. A. V. and Renart, C. M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), Food Polysaccharides and their Applications. 287-369. New York. Marcel Dekker.

Wagner, A. E. and Meeker, W.Q. jr., (1985), Preceding of the Statistical Computing Section, (Amer. State. Ass.: Washington, DC), P.441.

Waldron, K. W., Parker, M. L., Smith, A. C. (2003). Plant cell walls and food quality. Comprehensive Reviews in Food Science and Food Safety, 2, 101-119.

Wang, Q.; Pagan, J.; Shi, J., (2002) Pectin From Fruits. In Functional Foods: Biochemical and Processing Aspects, Shi, J.; Mazza, G.; Le Maguer, M., Eds. CRC Press LLC: Washington, 2002; Vol. 2

Whistler, R. L. and Be Miller, J. N.(1997). Pectins. In Carbohydrate Chemistry for Food Scientists (pp.203-210). St. Paul, MN: Eagan Press.

Willats, W. G. T., Knox, J. P., Mikkelsen D. J. (2006). Pectin: new insights into an old polymer are starting to gel. *Trends in Food Science & Technology, 17*, 97.

Zhemerichkin, D. A.; Ptitchkina, N. M. (1995)The composition andproperties of pumpkin and sugar beet pectins.Food Hydrocol-loids, 9, 147- 149.