SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF GRADUATE STUDIES

EFFECTS OF FREEZING PROCESS AND FROZEN STORAGE ON THE NUTRITIONAL VALUE OF SOME VEGETABLE PRODUCTS

أثر عملية التجميد والتخزين المجمد في القيمة الغذائية لبعض منتجات الخضروات

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إقرار

أنا الموقع أدناه أُقر بأننى المؤلف الوحيد لرسالة الدكتوراه المعنونة أثر عملية التجميد والتخزين المجمد في القيمة الغذائية لبعض منتجات الخضروات

وهى منتج فكري أصيل . وباختياري أعطى حقوق طبع ونشر هذا العمل لكلية الدراسات العليا جامعه السودان للعلوم والتكنولوجيا ،عليه يحق للجامعه نشر هذا العمل للأغراض العلمية .

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بسم الله الرحمن الرحيم

(أَنَا صَبَبنَا المَاءَ صَبَاَ * ثُمَ شَقَقنَا الأَرضَ شَقَاَ * فَأَنبَتنَا فِيهَا حَبَاَ * وَعِنَبَاَ وَقَضبَاَ * وَزَيتُونَاَ وَنَخلَاَ * وَحَدَائِقَ غُلبَاَ * وَفَكِهَةً وَأَبَاَ * مَتَعاً لَكُم وَلِأَنعَمِكُم)

صدق الله العظيم

سورة عبس
ايات 25-32

DEDICATION

To:

Soul of my great father Professor Dr. Nuri and my wonderful mother Aisha

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LIST OF ABBREEVIATIONS

kcal	Kilo Caloric
kj	Kilo Joule
kg	Kilo Gram
g	Gram
mg	Milligram
ml	Millilitre
min	Minute
sec	Second
nm	Nanometer
rpm	Revolutions per minute
°C	Degrees Celsius
β	Beta
TAG	Triacylglycerides
ROS	Reactive Oxygen Species
$^1O_2$	Single Oxygen
·OH	Hydroxyl Radical
O_2.-	Superoxide
HTST	High Temperature Short Time
ORAC	Oxygen Radical Absorbance Capacity
IQF	Individual Quick Freezing
EDTA	Ethylene Diamine Tetra-acetic Acid
TAF	Total Folate Activity
SPSS	Statistical Package for Social Science
ANOVA	Analysis of Variation
DMRT	Duncan's Multiple Range Test

ABSTRACT

The main goal of this study was to investigate the effects of freezing process and frozen storage on the nutritional value of some fresh vegetables products namely; okra, green beans, peas, spinach and molokhia. The results obtained in this study indicated that, all the investigated vegetables contain high levels of moisture, available carbohydrates and crude fiber which ranged between 83.86 - 86.52%, 4.13 - 6.83% and 5.64 - 6.42%, respectively, but, with very low levels of protein, fat and energy which ranged between 1.42 - 1.93%, 0.77 - 0.87%, and 27.66 - 39.70 kcal/100g, respectively. Also, all the vegetables were found very rich in potassium, magnesium, calcium, sodium and ascorbic acid which ranged between 596.81 - 823.83, 564.45 - 783.26, 208.65 - 603.57, 127.03 - 269.42 and 38.13 - 56.32 mg/100g, respectively. Moreover, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes were found at the maximum levels (100%) in the different fresh vegetables.

On the other hand, after the blanching process, the moisture content increased slightly by 1.43%, 1.70%, 1.84%, 1.98% and 2.25% in green beans, molokhia, spinach, peas and okra, respectively. While, the total carbohydrates, energy value, ascorbic acid, sodium, potassium, calcium, magnesium contents and the activity of peroxidase, lipoxygenase and polyphenol oxidase enzymes were significantly decreased. The decrease as per-cent was estimated to range between 5.64 - 10.13%, 2.04 - 11.76%, 11.61 - 20.37%, 11.42 - 26.59%, 9.53 - 20.79%, 11.08 - 25.79%, 90.03 - 91.32%, 90.03 - 91.46% and 90.08 - 90.97%, respectively.

After the freezing processing step, the moisture content in the different vegetables slightly decreased and therefore, the energy value and the available carbohydrates, increased by 6.02 - 16.23% and 10.61 - 26.91%, respectively. While, sodium, potassium, calcium, magnesium and ascorbic acid contents continued to decrease by 8.44 - 16.76%, 3.00 - 11.49%, 3.27 - 13.53%, 0.87 -

1.62% and 4.59 - 14.35%, respectively in comparison with their values after the blanching step. In addition to that, the freezing processing step slightly affected the activity of peroxidase, lipoxygenase and polyphenol oxidase enzymes.

After a frozen storage period of 12 months at -18°C, the energy values and available carbohydrates in the different vegetables gradually increased during the frozen storage period. The increment as per-cent by the end of the period was estimated to range between 19.75 - 20.54% and 29.90 - 48.28%, respectively. Whereas, sodium, potassium, calcium, magnesium and ascorbic acid in the different vegetables were decreased by 8.12 - 23.02%, 2.91 - 8.19%, 8.60 - 18.36%, 5.19 - 13.82% and 3.46 - 4.83%, respectively by the end of the frozen storage period compared to their values after the freezing processing step (zero time). Moreover, the enzymes inactivation in the different vegetables as per-cent by the end of the frozen storage period was estimated to range between 33.33 - 40.79%, 31.95 - 42.96%, and 28.85 - 37.20%, for peroxidase, lipoxygenase and polyphenol oxidase enzymes, respectively.

ملخص الأطروحة

الهدف الاساسي لهذه الدراسة هو دراسة اثر عمليتي التجميد والتخزين المجمد على القيمة الغذائية لبعض منتجات الخضروات الطازجة وهي بالتحديد البامية، الفاصوليا الخضراء، البسلة، السبانخ والملوخية. اشارت النتائج المتحصل عليها في هذه الدراسة ان جميع الخضروات تحت الدراسة تحتوي على مستويات عالية من الرطوبة، الكربوهيدرات المتاحة والالياف حيث تراوحت ما بين 83.86 - 86.52%، 4.13 - 6.83% و5.64 - 6.42%، على التوالي، إلا انها تحتوي على مستويات قليلة جداً من البروتين، الرماد، الدهون والطاقة حيث تراوحت ما بين 1.42 - 1.93%، 0.77 - 0.87%، 0.43 - 0.57% و27.66 - 39.70 كيلوجول/100جم، على التوالي. كذلك وجدت جميع الخضروات بانها غنية جداً في محتوها من البوتاسيوم، الماغنسيوم، الكالسيوم، الصوديوم وحمض الاسكوربيك والتي تراوحت ما بين 596.81 - 823.83، 564.45 - 783.26، 208.65 - 603.57، 127.03 - 269.42 و38.13 - 56.32 ملجم/100جم، على التوالي. بالإضافة إلى ذلك وجدت انزيمات البيروكسيديز، الليبواكسجينيز والبوليفينول اكسيديز على مستوى عالي من النشاط (100%) في جميع الخضراوات الطازجة.

من جهة اخرى، بعد خطوة عملية السلق ارتفعت نسبة الرطوبة قليلا بنسبة 1.43%، 1.70%، 1.84%، 1.98% و2.25% في كل من الفاصوليا الخضراء، الملوخية، السبانخ، البسلة والبامية، على التوالي. بينما كان هناك انخفاضاً معنوياً في قيمة كل من الكربوهيدرات الكلية، قيمة الطاقة، حمض الاسكوربيك، الصوديوم، البوتاسيوم، الكالسيوم، الماغنسيوم ونشاط كل من انزيم البيروكسيديز، الليبواكسيجينيز والبوليفينول اكسيديز. ولقد تراوحت نسبة هذا الانخفاض ما بين 5.64 - 10.13%، 2.04 - 11.76%، 11.61 - 20.37%، 11.42 - 26.59%، 9.53 - 20.79%، 11.08 - 25.79%، 12.31 - 21.72%، 90.03 - 91.32%، 90.03 - 91.46%، و90.08 - 90.97%، على التوالي.

لكن، بعد خطوة عملية التجميد انخفضت نسبة الرطوبة قليلاً في جميع الخضراوات المختلفة وبناءاً على ذلك ارتفعت قيمة الطاقة والكربوهيدرات المتاحة بنسبة 6.02 - 16.23% و10.61 - 26.91%، على التوالي، بينما استمرت قيم كل من الصوديوم، البوتاسيوم، الكالسيوم، الماغنسيوم و حمض الاسكوربيك في الانخفاض بنسبة 8.44 - 16.76%، 3.00 - 11.49%، 3.27 - 13.53%، 0.87 - 1.62% و4.59 - 14.35%، على التوالي بالمقارنة مع قيمها بعد خطوة عملية السلق. بالاضافة إلى ذلك لقد أثرت عملية التجميد قليلا على نشاط انزيمات البيروكسيديز، الليبواكسيجينيز والبوليفينول اكسيديز.

إيضاً، بعد التخزين المجمد لمدة 12 شهر عند درجة حرارة -18°م، ارتفعت قيمة الطاقة والكربوهيدرات المتاحة تدريجيا أثناء فترة التخزين. ولقد تراوحت نسبة هذا الارتفاع في نهاية فترة التخزين

المجمد ما بين 19.75 - 20.54% و29,90 - 48.28%، على التوالي. بينما انخفضت نسبة كل من الصوديوم، البوتاسيوم، الكالسيوم، الماغنسيوم وحمض الاسكوربيك بنسبة 8.12 - 23.02%، 2.91 - 8.19%، 8.60 - 18.36%، 5.19 - 13.82% و3.46 - 4.83%، على التوالي بنهاية فترة التخزين المجمد مقارنة بقيمها بعد خطوة عملية التجميد (الزمن صفر) عند بداية فترة التخزين المجمد. بالاضافة إلى ذلك لقد قدرت نسبة تثبيط الانزيمات عند نهاية فترة التخزين المجمد كنسبة مئوية ما بين 33.33 - 40.79%، 31.95 - 42.96%، و28.85 - 37.20% لكل من انزيم البيروكسيديز، الليبواكسيجينيز والبوليفينول اكسيديز، على التوالي.

CHAPTER ONE

1. INTRODUCTION

1.1 Vegetables and freezing process

Vegetables have long been known as a nutritious and healthful part of the human diet, because they are low in calories, protein and fat, but are very rich in vitamins, minerals, and fibers. In addition to that, vegetables are also, rich in phenolic compounds such as anthocyanins and flavanoids, which have been found to correlate with lower risks of chronic diseases. But, the real challenges have always been how to find means and ways to preserve these food with high quality until thus reach the consumer **(Mullen *et al.*, 2002; Chaovanalikit and Wrolstad, 2004)**.

Freezing as a processing technique is considered as one of the oldest and most widely used method for food preservation, which allows better preservation of taste, texture and food nutritional values in comparison with the other preservation methods. Also, the freezing process has been successfully employed for long-term preservation of many foods, providing a significantly extended shelf-life **(Chaovanalikit and Wrolstad, 2004)**.

Moreover, from a technical point of view, the freezing process is considered as the most convenient and easiest method of food preservation, compared with the other commercial preservation techniques. Also, the low capital investment of the freezing industry usually plays an important role in terms of economic feasibility of the process in the developing countries, as the energy consumption during the freezing process and frozen storage constitute approximately 10 percent of the total production cost **(Péneau, 2005)**.

In fact, the future growth of frozen foods will mostly be affected by population growth, personal incomes, relative cost of other forms of food, changes in tastes and preferences and the technological advances of the freezing methods **(Barbosa-Cánovas *et al.*, 2005)**.

The main concept of this process involves lowering the product temperature generally to -18°C or below and maintaining it during the storage period. According to **Barbosa-Cánovas *et al.*, (2005)**, freezing technology is a combination of beneficial effects of low temperature at which microorganisms cannot grow, chemical as well as cellular metabolic reactions will be reduced. Therefore, the process is considered superior to canning and dehydration technologies when the retention of sensory and nutritional properties are considered **(Krinsky, 2005)**.

The use of freezing technology allows the retention of vegetables freshness and extending their availability for long period as vegetables are well known as highly perishable foods and could be subjected to rapid deterioration by microorganisms, enzymes, or oxidation reactions **(Barbosa-Cánovas *et al.*, 2005)**. Safety and high nutritional value of frozen products are usually achieved when high quality raw materials are used with good manufacturing practices during the preservation process. In addition to that, the products should be kept in accordance with the specified storage temperatures.

Today an increasing demand for frozen foods already exists and further expansion of the industry is primarily dependent on the ability of food processors to develop high qualities in both process techniques and products. Therefore, efforts should be focused on new technologies and factors that will improve the nutritive values of frozen foods products **(Flores *et al.*, 2010)**.

1.2 Aim of the study

1.2.1 Main objective

The main goal of this study is to investigate the effects of freezing process and frozen storage on the nutritional value of some vegetables products namely; okra, green beans, peas, spinach and molokhia which are usually exported from Egypt to European countries.

1.2.2 Specific objectives

1) To investigate the nutritional value of fresh vegetable products with respect to their chemical composition, minerals and vitamins.
2) To study the effects of blanching and freezing processes on their nutritional values.
3) To determine the effects of frozen storage conditions on the nutritional values of the frozen vegetable products.
4) To study the effects of the frozen storage conditions on vegetables enzymes activity and hydrogen ions concentration (pH).
5) To determine maximum shelf-life of the frozen vegetable products.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Vegetables

2.1.1 Relative importance

Vegetables are considered as very essential food for human nutrition and health. In addition to their major nutrients such as carbohydrates, protein, fat, and minerals, vegetables are also, abundant in a number of essential phytochemicals compounds such as ascorbate, carotenoids, phenolics, glucosinolates and phytosterols **(Lampe, 1999; Kris-Etherton *et al.*, 2002)**. Some of these phytochemicals possess antioxidant activity as well as antimicrobial or hormonal activities. Vegetables are very rich in ascorbic acid, β - carotene, and contain lower amounts of trypsin inhibitors, phytates, and oligosaccharides. Vegetables are also, rich in fiber, and provide significant amounts of iron, potassium, magnesium and Vitamin-C **(Song *et al.*, 2003)**.

Numerous epidemiological studies have shown that a diet rich in vegetables has beneficial effects on human health that reduce the risk of certain diseases like cardiovascular disease, heart disease, Alzheimer's disease and some forms of cancers **(Kang *et al.*, 2005)**. Moreover, vegetables are considered as one of the few natural sources of isoflavones such as genistin and daidzein. Tocopherols are also, present in vegetables **(Kamga *et al.*, 2013)**.

2.1.2 Nutritional value

2.1.2.1 Protein

As mentioned by **Young (1991),** vegetables protein meets the protein needs of human adults, when consumed as a sole source of protein. Vegetables contain most of the essential amino acids required for human and animal nutrition; isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptohpan, valine, and histidine **(Carter and Shanmugasundaram,**

1993). Among all of the plant protein sources, vegetables provide the most complete amino acid balance for human and animal food **(Cui *et al.*, 1999)**. Vegetables proteins provide amino acids equal or in excess to human requirements **(Sahreen *et al.*, 2010)**.

2.1.2.2 Lipid

Liu (1997) indicated that, the vegetables lipid is primarily made up of triacylglycerides (TAG). TAG is neutral lipids consisting of three fatty acids attached to one glycerol molecule. Similar to many other plant oils, the majority of fatty acids in vegetables are unsaturated. The most abundant fatty acid in vegetables is linoleic acid, followed in descending order by oleic, palmitic, linolenic, and stearic acid. Consumption of monounsaturated (oleic) and polyunsaturated (linoleic and linolenic) fatty acids is negatively correlated with risk of cardiovascular disease. The consumption of these fatty acids was found to decrease the total cholesterol and improve the cholesterol lipoprotein ratios **(Groff and Gropper, 2000; Lawless and Heymann, 2010)**.

2.1.2.3 Carbohydrates

The soluble carbohydrates found in vegetables are primarily disaccharides (sucrose) and oligosaccharides such as raffinose, and stachyose **(Liu, 1997)**. Humans do not have the ability to digest oligosaccharides in the duodenal or small intestinal mucosa **(Groff and Gropper, 2000)**. Consumption of raffinose and stachyose may result in significant amounts of these sugars passing unabsorbed into the colon where they are then metabolized by the colon bacteria and result in gas production **(Mahan *et al.*, 2000)**.

Also, vegetables contain insoluble carbohydrates such as cellulose, hemicellulose, pectin, and trace quantities of starch. Consumption of insoluble carbohydrates is recommended for humans, as they increase the fecal weight, delay

the digestion of starch and slower the absorption of glucose in the blood stream **(Groff and Gropper, 2000)**. Both the soluble and insoluble carbohydrates in vegetables fall under the category of dietary fiber. Fiber consumption has been linked to optimum health and diseases resistance. Soluble fiber has been found to lower the density of lipoprotein of total serum cholesterol, while insoluble fibers exert an overall protective effect and reduce cancer risk **(Mahan *et al.*, 2000; Kamga *et al.*, 2013)**.

2.1.2.4 Vitamins

Vitamin-C (ascorbic acid or dehydroascorbic acid) is an important nutritional component in many horticulture crops. It has great beneficial effects on cellular mechanisms including collagen formation, absorption, immunity, and antioxidant capabilities **(Mahan *et al.*, 2000)**. More than 90% of the vitamin in human diets is supplied by vegetables. The oxidation of ascorbic acid is favorable in the presence of oxygen, heavy metal ions (copper and iron), alkaline pH and high temperature. In addition to that, the losses are enhanced in vegetable products under extended storage at elevated temperature, low relative humidity, physical damage, and chilling injury. The blanching step may also, reduce the vitamin-C content during the process, but successfully the losses declined during frozen storage of vegetable products **(Lee and Kader, 2000)**.

During processing, distributing, and storage of frozen vegetables, ascorbic acid oxidizes to dehydroascorbic acid which is irreversibly hydrolyzed and possesses no vitamin-C activity **(Giannakourou and Taoukis, 2003)**.

Ascorbate is synthesized in plants through three different pathways and it has been characterized as a highly effective antioxidant, an enzyme co-factor for the biosynthesis of many biochemicals as well as an electron donor/acceptor. Also, it acts as a precursor in the formation of organic acids in plants and is also involved

in the regulation of cell division and elongation **(Davey *et al.*, 2000; Valpuesta and Botella, 2004 ; Debolt *et al.*, 2007)**.

Ascorbate is a common metabolite in plants, but it is an essential nutrient in humans as it cannot be biosynthesized in human body. Vitamin-C is required for the synthesis of collagen and neurotransmitter, and also has other biological activities in human body such as protecting plasma lipids through its radical scavenging activity as well as enhancing the immune system **(González *et al.*, 2005)**. It is often regarded as quality indicator for fresh vegetables during postharvest storage **(Podsędek, 2007)**. Vitamin-C deficiency causes physiological disorders such as scurvy and in extreme cases can lead to morbidity **(Lawless and Heymann, 2010)**. Tables (2.1), (2.2), (2.3), (2.4) and (2.5) show the nutritive values of raw and frozen okra, green beans, peas, spinach and molokhia, respectively, as reported by **USDA (2012)**.

Table (2.1): Nutritive value of raw and frozen okra per 100g

Ingredients	Raw okra	Frozen okra
Calories	31	30
Protein (g)	2	2
Fat (g)	0	0
Carbohydrate (g)	7	7
Fiber (g)	3	2
Sodium (mg)	8	3
Calcium (mg)	8	8
Iron (mg)	4	3
Vitamin C, ascorbic acid (mg)	35	21
Vitamin A, Carotenoids (mg)	7	7

Table (2.2): Nutritive value of raw and frozen green beans per 100g

Ingredients	Raw green beans	Frozen green beans
Calories	31	39
Protein (g)	2	2
Fat (g)	0	0
Carbohydrate (g)	7	8
Fiber (g)	3	3
Sodium (mg)	6	3
Calcium (mg)	4	4
Iron (mg)	6	5
Vitamin C, ascorbic acid (mg)	27	22
Vitamin A, Carotenoids (mg)	14	11

Table (2.3): Nutritive value of raw and frozen peas per 100g

Ingredients	Raw peas	Frozen peas
Calories	81	77
Protein (g)	5	5
Fat (g)	0	0
Carbohydrate (g)	14	14
Fiber (g)	5	5
Sodium (mg)	5	3
Calcium (mg)	2	2
Iron (mg)	8	9
Vitamin C, ascorbic acid (mg)	67	30
Vitamin A, Carotenoids (mg)	75	41

Table (2.4): Nutritive value of raw and frozen spinach per 100g

Ingredients	Raw spinach	Frozen spinach
Calories	23	29
Protein (g)	3	4
Fat (g)	0	0
Carbohydrate (g)	4	4
Fiber (g)	2	3
Sodium (mg)	9	4
Calcium (mg)	10	13
Iron (mg)	15	10
Vitamin C, ascorbic acid (mg)	47	29
Vitamin A, Carotenoids (mg)	38	23

Table (2.5): Nutritive value of raw and frozen molokhia per 100g

Ingredients	Raw molokhia	Frozen molokhia
Calories	46	42
Protein (g)	1	1
Fat (g)	0	0
Carbohydrate (g)	7	6
Fiber (g)	1	1
Sodium (mg)	4	4
Calcium (mg)	12	12
Iron (mg)	15	15
Vitamin C, ascorbic acid (mg)	50	43
Vitamin A, Carotenoids (mg)	38	31

2.1.3 Vegetables bioactive compounds

2.1.3.1 Effects of vegetables bioactive compounds on human health

As reported by **Finkel and Holbrook (2000) and Hodges (2003)**, the reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), single oxygen ($^1O_2$), hydroxyl radical (·OH) and superoxide (O_2.-) are produced in the respiratory chain in the mitochondria as by-products during normal cellular metabolism. Both biotic (e.g. pathogens) and abiotic factors (e.g. extreme temperature, light, storage duration, processing methods and conditions accelerating water loss or/and ripening) can trigger excess production of ROS under growth and post-harvest conditions. Excessive ROS was found to react with various cellular components such as lipids, carbohydrates, proteins and nucleic acids, leading to lipid peroxidation, protein denaturation and mutagenesis.

Epidemiological studies also revealed that whole vegetables were more efficient than its purified chemical component in reducing the risk of diseases due to the interaction between bioactive components in the whole vegetables **(Holick *et al.*, 2002)**.

The relationship between a high risk of cardiovascular disease or certain kinds of cancer with a low intake of β-carotene, or flavonoids has been studied **(Neuhuser, 2004; Arts and Hollman, 2005)**. Higher concentration of antioxidants at harvest was found to ensure the ability of vegetables to reduce oxidative stress during subsequent storage **(Hodges *et al.*, 2004)**.

Bergquist *et al.* (2006) found that, vegetables when harvested a few days earlier had higher ascorbic acid, exhibited improved visual quality and better nutritional value during storage. Besides, the positive effects on human health, the bioactive compounds themselves may affect the shelf life of fresh product **(Sipos *et al.*, 2009)**.

However, more detailed and unbiased intervention trials are needed to measure the beneficial effects and/or the optimum dosage of certain

phytochemicals on humans **(Sahreen *et al.*, 2010)**. Moreover, many phytochemicals have recondite effects on human health which is dependent on their doses and this may in part explain the conflicting findings from epidemiological studies **(Gil-Chávez *et al.*, 2013)**.

Gil-Chávez *et al.* (2013) mentioned that, increasing the content of bioactive compounds in vegetables at harvest may not only have beneficial effects on human health, but may also improve the appearance, prolong shelf life and reduce post-harvest losses of fresh produce.

Thus, antioxidative phytochemicals in vegetables such as carotenoids and polyphenols provide protective effects against ROS damage **(Ioannou and Ghoul, 2013)**.

2.1.3.2 Factors affecting bioactive compounds in vegetables

2.1.3.2.1 Genetic variation

The flavonoid glycosides were identified in some vegetables and not present in other vegetables **(Bergquist *et al.*, 2005)**. Some of the bioactive compounds in vegetables are ubiquitous, while others are unique to specific families, species or even cultivars. Ascorbic acid and phenolic compounds are found in many vegetables but the concentrations vary among them **(Kevers *et al.*, 2007; Lin and Tang, 2007)**. Flavonoid content has been shown to differ among vegetables genotypes **(Cho *et al.*, 2008)**. Thus, genetic factors have significant influences on the composition of bioactive compounds in fresh vegetables products.

2.1.3.2.2 Pre-harvest factors

Veit *et al.* (1996) indicated that, the harvest time during the day may result in significant changes in the concentrations of bioactive compounds, possibly related with light intensity and water content. A number of pre-harvest factors such as temperature and light intensity during growth, water supply and soil

characteristics were found to affect the concentration of bioactive compounds in vegetables **(Weston and Barth, 1997; Ferguson *et al.*, 1999)**. Also, **Lefsrud *et al.* (2005) and Bergquist *et al.* (2007a and 2007b)** reported that, the environmental conditions such as air, temperature and light condition affect the chemical composition in vegetables. Shade netting decreased ascorbic acid concentration but increased the carotenoid content in vegetables.

Growth stage and maturity were found also, to affect the concentration of bioactive compounds. Mid-mature vegetables have higher total phenolics and flavonoids than immature and over ripe vegetables **(Pandjaitan *et al.*, 2005)**. However, carotenoids and/or flavonoids accumulate during vegetables ripening to provide color to the ripe vegetables **(Kalt, 2005)**. In contrast, the highest ascorbic acid content was found in immature vegetables **(Bergquist *et al.*, 2006)**. **Reyes *et al.* (2007)** reported that mechanical injuries at harvest may also increase antioxidant content in fresh produce.

2.1.3.2.3 Post-harvest factors

The post-harvest quality of vegetables was reported to be influenced by method of harvesting, CO_2 and O_2 partial pressure, ethylene, water vapour pressure, microbial decay, bruising, storage temperature and duration of storage. Micro-organisms as well as insect pests were also resulted in considerable postharvest loss of fresh products **(Kader, 2002)**. Chemical treatments such as cytokinin and ethylene application, soaks of exogenous antioxidants and coatings of edible materials which exert physical protection are also employed to reduce oxidative-associated injury **(Toivonen, 2003 and Toledo *et al.*, 2003)**.

Ascorbic acid was found to decrease rapidly during storage in many plant products while flavonoids and carotenoids were generally stable **(Kalt, 2005; Kevers *et al.*, 2007)**. A lower storage temperature (2°C) resulted in a smaller reduction in both visual quality and ascorbic acid content of vegetables, probably

due to the decrease in metabolic rates (**Hodges *et al.*, 2004; Bergquist *et al.*, 2006**). Controlled atmosphere storage has also been shown to influence antioxidant levels. For example; low O_2 levels impeded senescence by slowing down the rate of oxidative respiration in the mitochondria (**Tripathi and Dubey, 2004; Hodges and DeLong, 2007**).

Postharvest losses result from physiological changes accelerated by abusive conditions such as improper storage conditions, low atmospheric humidity and high temperatures (**Meng *et al.*, 2008; Sahreen *et al.*, 2010**). Unfavourable storage conditions were found to induce oxidative stress (**Retamales *et al.*, 2003; Gil-Chávez *et al.*, 2013**).

2.1.4 Effects of freezing processing steps on the nutritional values of vegetable products

2.1.4.1 Effects of pre-freezing treatments

2.1.4.1.1 Effects of peeling

As mentioned by **Thane and Reddy (1997)**, some vegetables should be peeled before processing by using hot water, hot sodium hydroxide solution or mechanical peelers. The process may remove some of the nutrients with the portions that are separated from the peeled products. Also, after peeling the flesh of the vegetable exposed to the atmosphere and therefore some carotenoid may be lost through oxidation.

According to **Van den Berg *et al.* (2000)**, the nutrients losses during the freezing process may be due to physical separation (peeling and trimming) prior to freezing or during thawing, leaching during blanching or chemical degradation during storage.

2.1.4.1.2 Effects of blanching

2.1.4.1.2.1 Vitamins and minerals

Masuda (1991) determined a direct relationship between the decline in ascorbic acid and browning of vegetables, during refrigerated storage. **Wu *et al.* (1992)** reported a reduction of about 14% and 17%, of ascorbic acid in vegetables during blanching for 3 min in boiling water, on dry and wet basis, respectively.

According to **Brewer *et al.* (1994)**, the loss of water-soluble minerals and vitamins during blanching should be minimized by keeping blanching time and temperature at an optimum combination. **Brewer *et al.* (1994 and 1995)** studied the effect of steam, water, and microwave blanching on the retention of ascorbic acid in vegetables. The steam blanching method was found to be the best.

Van den Berg *et al.* (2000) compared the effects of water and high temperature short time (HTST) steam blanching methods on vegetables ascorbic acid content before and after the treatment. After blanching, the vegetables were frozen and stored for 90 days at -23°C. The authors found that, vegetables treated with water (82°C for 3 min) and steam did not show differences in their ascorbic acid contents, except for those treated at 45 psi for 35 sec, resulted in less destruction of ascorbic acid.

Murcia *et al.*, (2000) also, studied the effect of hot water blanching method on vegetables. Water blanching of vegetables at 92-96°C for 60-150 s resulted in a loss in ascorbic acid varying from 50- 55%, depending upon the treatment. Blanching can also lead to thermally induced degradation of nutrients, such as ascorbic acid. Ascorbic acid losses increase with extended storage, improper frozen storage temperatures, low relative humidity, physical damage, and chilling injury **(Lee and Kader, 2000)**.

The retention of ascorbic acid in frozen products is dependant of the product temperature history **(Giannakourou and Taoukis, 2003)**; including harvesting conditions, processing parameters, and temperatures during frozen storage.

Puupponen-Pimiä *et al.* (2003) found that, the freezing step did not affect the nutritional value of the vegetables and all the differences observed between the raw and frozen product attributed to the blanching step. **Puupponen-Pimiä *et al.* (2003)** studied the effect of blanching on the folate content of vegetables. The author reported losses of 83% and 42% for water and steam blanching in folic acid, respectively.

Steam blanching was found to take longer time than the hot water method, but it was mentioned to retain water-soluble nutrients such as some vitamins and minerals. After blanching, the product should be rapidly cooled down to minimize the degradation of heat-labile nutrients **(Barbosa-Cánovas *et al.*, 2005)**.

Sipos *et al.* (2009) also, showed a loss of 4-10% in the thiamin content and 7-18% in the riboflavin content of vegetables during blanching at 96-98°C for 2.5-4.0 min. The highest loss in ascorbic acid (30%) occurred in vegetables which had the largest inherent surface area per unit weight. Blanching was mentioned to be carried out by using hot water, steam and microwave (75 and 95°C) for 1 to 10 minutes, depending on the size of individual vegetable pieces. The same author found that, the loss of water-soluble vitamins during water blanching of vegetables increased with contact time, while fat-soluble vitamins are relatively unaffected. In contrast, steam blanching resulted in a greater retention of water-soluble vitamins in comparison with water blanching method. The longer the blanching time of vegetables the lower the retention of ascorbic acid in the final product. The data obtained for the effect of blanching on thiamine showed that for most of the treatments, the loss after blanching was less than 10% of the thiamine in raw vegetables.

Calcium and magnesium which are generally bound to the plant tissue are not readily lost by leaching and sometimes can even be taken up by vegetables during blanching when hard water is used. Potassium, the most abundant mineral

in vegetables (**Sipos *et al.*, 2009**), is extremely mobile and is easily lost by leaching during blanching because of its high solubility in water (**Xu *et al.*, 2012**).

2.1.4.1.2.2 Antioxidants

The effect of blanching conditions on the amounts of phenolic compounds was studied by **Puupponen-Pimiä *et al.* (2003)**. All the differences observed in the final product with regard to their nutrients content were attributed to the blanching step. The authors suggested that the observed increase in fiber content was a result of washing out of soluble solids and consequently increased the fiber content in the frozen product.

In general, the amount of total phenolics decreased during blanching by 20-30%. **Ninfali and Bacchiocca (2003)** studied the effect of blanching and freezing on the amount of polyphenols and antioxidant capacity of vegetables. The authors reported that the amount of phenols and oxygen radical absorbance capacity (ORAC) values in frozen vegetables were about 30 and 12%, respectively, in comparison with those observed in the fresh vegetable. Frozen vegetables, did not show differences in oxygen radical absorbance capacity value when compared with fresh vegetable, indicating that the blanching method was mild enough to preserve the antioxidant capacity of the product (**Czarnowska and Gujska, 2012**).

2.1.4.1.2.3 Pigments

Van den Berg *et al.* (2000) explained the higher carotenoid content in blanched vegetables. **Puupponen-Pimiä *et al.* (2003)** reported an increase in concentration of carotenoids content after blanching and freezing.

Scott and Eldridge (2004) reported a significant increment in the level of β-carotene in vegetables after blanching and freezing (on wet basis), and attributed the increases observed to a possible dehydration of the vegetables during steam blanching and freezing.

A further effect of thermal processing is the degradation of chromophores such as chlorophyll, resulting in color change. Pigment degradation will continue to take place in frozen storage **(Savas *et al.*, 2005)**. Vegetables color has been directly correlated with ascorbic acid content.

2.1.4.1.2.4 Physical and chemical characteristics

In the frozen vegetable industry sometimes quality has been a second priority due to over compensation in the area of food safety **(Barrett and Theerakulkait, 1995)**. Consumers demand that frozen vegetable products have a high resemblance to the raw counterpart, which requires minimal blanching induced degradation of nutritional and organoleptic properties **(Ludikhuyze *et al.*, 1998)**.

Texture is one of the main attributes that define the quality of preserved vegetables, with lack of firmness being a limiting factor in marketing and consumer acceptability **(Young *et al.*, 2000)**. Heating produces micro-structural alterations in plant tissue that influence texture. The general result is softening, brought about by loss of turgor pressure and occluded air, thermal degradation of middle lamella-pectins and other cell wall polysaccharides and starch gelatinization **(Llano *et al.*, 2003)**.

The texture of vegetables has been previously studied in relation to consumer acceptance **(Song *et al.*, 2003)**. The consumers were found to prefer vegetables which had a soft texture. **Song *et al.* (2003)** recommended blanching vegetables by using the high temperature short time (HTST) approach.

Sensory evaluation and instrumental determination are two objective methods to measure product texture **(Hansen *et al.*, 2004)**. Force/deformation methods are commonly used in instrumental analysis. These direct methods measure single or multiple mechanical properties of food, which are important in the sensory perception of texture by humans. Within the force/deformation

analysis, a destructive approach to measuring texture is the compression method. The destructive methods are preferred means for measurement of food texture because they highly correlate with the sensory evaluation methods **(Abbott and Abbott, 2004)**. Compression can be used to measure solid foods and is considered to be qualified for prediction of sensory perception of consistency during mastication **(Hansen *et al.*, 2004)**.

The majority of vegetables require a short heat treatment for blanching before freezing **(Martinis and Silva, 2004)**. Blanching of vegetables prior to freezing has both advantages and disadvantages. Given that, during blanching changes associated with mild thermal processing can be expected. Blanching should provide uniform heat distribution to the individual product, uniform blanching time, overall high quality, high product yield, low consumption of energy and water **(Savas *et al.*, 2005)**.

The blanching process can be achieved by immersing vegetables in boiling water **(Matkowski, 2009)**. If vegetables are stored in frozen conditions immediately following harvesting, there is a notable decline in quality **(Udousoro *et al.*, 2013)**.

2.1.4.1.2.5 Enzymatic reactions

Blanching as a pre-freezing treatment is used to inactivate enzymes that cause undesirable changes in color, flavor, and nutritive value during frozen storage **(Murcia *et al.*, 2000)**. Conventionally, vegetables are blanched to the point of inactivation of specific enzymatic (e.g. Lipoxygenase) activity **(Martinis and Silva, 2004)**. Adequate blanching should provide inactivation of enzymes, improved microbial status, shortened cooking time for the finished product, and stabilization of texture, flavor and nutritional quality. Enzymatic activity will continue in un-blanched vegetables at low temperatures. This enzymatic activity may result in changes in texture, flavor, color, and nutrients content. Blanching is a

thermal process designed to inactivate the enzymes responsible for generating off flavors and odors. Lipoxygenase, lipases, and proteases have been associated with off flavor development, while, pectic enzymes and cellulases have been shown to cause textural changes. Also, polyphenol oxidase, chlorophylase, and peroxidase may cause color changes, whereas, ascorbic acid oxidase and thiaminase can lead to nutritional deterioration **(Savas *et al.*, 2005)**.

In secondary reactions, lipoxygenase produces lipid hydroperoxides and hydroperoxy radicals that affect chlorophyll and carotenoids and cause a loss of color **(Matkowski, 2009)**. A delicate balance between inactivating enzymes associated with degradation and minimizing losses in quality caused by prolonged blanching must be achieved in order to produce the highest quality frozen product **(Czarnowska and Gujska, 2012)**.

2.1.4.2 Effects of freezing step

2.1.4.2.1 Vitamins

Lisiewska and Kmiecik (1991) reported no any effect of freezing on the contents of thiamin and riboflavin of vegetables. **Selman (1992)** reported that, the process of freezing itself does not alter the nutritive value of the frozen product. **Cano *et al.* (1993)** studied the effect of freezing on vegetables. **De-Ancos *et al.* (2000)** showed that the freezing process had little effect on the total phenol, vitamin-C contents and antioxidant capacity on vegetables. The effect of freezing on total flavonoids and anthocyanins contents in vegetables were studied by **Mullen *et al.* (2002)**. Vegetables were frozen at -30°C without any additional treatments.

Ninfali and Bacchiocca (2003) indicated that, freezing by using a Lewis individual quick freezing (IQF) tunnel and blast freezers did not affect the ascorbic acid content of vegetables. **Scott and Eldridge (2004)** indicated that, the freezing

step had no significant effect on vitamins content of vegetables. As mentioned by **Sipos *et al.* (2009)**, during the freezing process the amount of ascorbic acid was decreased by 10-25%, but, the soluble solids, total and monosaccharides in the frozen vegetables did not change significantly. However, **Czarnowska and Gujska (2012)** reveal that, more vitamins losses occurred during the blanching and subsequent frozen storage period.

The total flavonols content of fresh vegetables was insignificantly different than in the frozen products (22.3 – 27.0 nmoles/g fresh weight). Also, insignificant differences in six major anthocyanin contents were noticed in the fresh and frozen vegetable products **(Garden-Robinson, 2013)**.

2.1.4.2.2 Pigments

According to **Thane and Reddy (1997)**, the amount of carotenoids were not affected by freezing, particularly rapid freezing. The deteriorative process occurred at a very low rate during storage. The vegetables were frozen in an air-blast freezer at -40°C without any previous treatments.

2.1.4.2.3 Physical and chemical characteristics

Freezing was found to preserve taste, texture, nutritional value of foods and extends food shelf life better than any other preservation method **(Giannakourou and Taoukis, 2003)**. Freezing also, decreases the rate of most deteriorative reactions such as senescence, enzymatic decay, chemical decay, and microbial growth **(Savas *et al.*, 2005)**.

Freezing has long been established as an excellent method for preserving food products, including vegetables. The total acidity of the vegetables was found to decrease slightly during the freezing process, in comparison with the raw vegetables **(Sipos *et al.*, 2009)**.

The freezing process includes pre-freezing treatments, freezing, frozen storage and thawing. The freezing process is regarded as the simplest and the most important preservation method for vegetables although some nutrients such as vitamins and minerals may be lost during the process (**Czarnowska and Gujska, 2012**).

2.1.4.3 Effects of frozen storage

2.1.4.3.1 Vitamins and minerals

During storage of some vegetables at -18°C for one year, the loss in the thiamin content was about 20%. Also, during freezing process and storage, ascorbic acid oxidized enzymatically to dehydroascorbic acid which has an equal antiscorbutic activity but, it is not stable and is hydrolysed to 2,3-diketogulonic acid and further breakdown products (**Selman, 1992**).

Wu *et al.* (1992) found that, the ascorbic acid content in vegetables was not changed when stored at -20°C for 16 weeks. But, after 30 months at -22°C, the vitamin-C content of frozen vegetables was reduced by 62.2%. Also, the effect of frozen storage (1 year) at - 18°C on the ascorbic acid content was studied by **Cano *et al.* (1993)**. **Favell, (1998)** found that after a storage period of 12 months at -20°C, the decrease in the ascorbic acid content of vegetables was less than 20%.

Lee and Coates (1999) showed that after 24 months of storage at -23°C, the loss in vitamin-C content in vegetables was about 19.2%. According to the authors, the estimated shelf-life of the product to meet the claimed levels of vitamin-C on the label could be about 22 months.

Frozen storage of vegetables for one year at -20°C showed a continuous decrease in vitamin-C with time, ranged between 33 and 55% at the end of the storage period. The decrease in reduced ascorbic acid during storage of frozen vegetables is partially compensated by the increase in dehydroascorbic acid (**De Ancos *et al.*, 2000**).

The same author also, studied the effect of storage at -15°C for up to 5 months on the composition of frozen vegetables. After 5 months of storage, significant losses were found in reduced ascorbic acid (31%) and total ascorbic acid, but the amount of dehydroascorbic acid was increased. Also, during the storage period the total acids were increased, while the pH decreased.

According to **Mullen *et al.* (2002)**, losses of vitamins-C, B1 and B2 during frozen storage are usually less in blanched than in unblanched vegetables. Also, the authors mentioned that a 10°C rise in temperature, within the range of -18 to -70°C, accelerates vitamin-C degradation by a factor of 6 to 20 times. About 50% loss in vitamin-C in the frozen vegetables took place after 26 months of storage, while 25% loss in vitamin-C occurred after about 13 months.

Puupponen- Pimiä *et al.* (2003) studied the effect of frozen storage on total folate activity (TFA) in vegetables. The retention of TFA in vegetables after 3 and 8 months of storage at -32.2°C was found to be 72% and 83%, respectively, when compared to the TFA in the raw products just after water-blanching before freezing. The author found that, storage of vegetables at -20°C for 18 months did not affect the amount of folic acid.

The effects of frozen storage (12 months) at -18°C on the levels of vitamins and minerals in vegetables concentrate were studied by **Sahari *et al.* (2004)**. Appreciable loss was recorded in ascorbic acid (50%), with variable decreases in folic acid, pantothenic acid, riboflavin, vitamin B6 and minerals. Significant decreases of 64.5%, 10.7%, and 8.9%, in ascorbic acid content after 90 days were found when the vegetables stored at -12, -18, and -24°C, respectively, with major losses occurring during the first 15 days of storage at -12°C (31.4%).

Increases in diketogulonic acid content between weeks 2 and 13 with a gradual decrease in reduced ascorbic acid were noticed in the products. Both dehydroascorbic acid and reduced ascorbic acid were biologically active. Storage of frozen vegetables at a temperature higher than -18°C for long periods of time

was found to cause vitamin losses and marked effects on color and flavor **(Czarnowska and Gujska, 2012)**. In fact, vitamin loss during frozen storage was found to depend on products, type of packaging and the utilization of additives or sugars **(Garden-Robinson, 2013)**.

2.1.4.3.2 Antioxidants

The levels of ellagic acid, a polyphenol antioxidant in numerous vegetables during frozen storage were studied by **De-Ancos *et al.* (2000)**. No major changes in total phenolic content was observed for one year at -20°C, while, the losses in the total ellagic acid content ranged between 14 - 21%. The authors attributed the decrease in ellagic acid content to a possible release of polyphenol oxidase enzyme from the cellular wall of the vegetables during storage.

The amount of total phenolics in vegetables was evaluated during frozen storage at -23°C and -70°C for 6 months by **Chaovanalikit and Wrolstad (2004)**. A degradation of 25% and 50% was reported after 3 months and 6 months at the higher and the lower storage temperature, respectively.

2.1.4.3.3 Pigments

The effect of freezing and frozen storage (-18°C for 12 month) on the carotenoids composition of vegetables was studied by **Cano *et al.* (1996)**. **De-Ancos *et al.* (2000)** did not observe any change in the monomeric anthocyanin content of vegetables stored at -20°C for up to 1 year. The results obtained showed a significant decrease in carotenoids content of frozen vegetables.

Puupponen-Pimiä *et al.* (2003) showed that during frozen storage of vegetables at -20°C for 18 month, 17% of β- carotene content was lost. Also, **Sahari *et al.* (2004)** reported a loss of 60% in vegetables carotenoids when stored for 12 months at -20°C.

Chaovanalikit and Wrolstad (2004) studied the effect of frozen storage in the anthocyanins content of vegetables. A degradation of 87% in anthocyanins was recorded when the vegetables stored at -23°C for 6 months. In contrast, the amount of polymeric color increased to 61% after frozen storage. But, when the vegetables stored at -70°C, 88% of anthocyanin remained after 6 months. Similar results were also, reported by **Hager *et al.* (2008)** for monomeric anthocyanin and polymeric color in individually quick frozen blackberries that stored for 6 months at -20°C.

2.1.4.3.4 Physical and chemical characteristics

Physical changes were observed in foods during mishandling, harvesting, processing, and distribution. Temperature, humidity, oxygen and light are environmental factors that can lead to physical changes in food during storage and distribution. These changes may alter food quality or becomes harmful to the consumer. The chemical changes associated with processed and stored foods are enzymatic, oxidative, and non-enzymatic reactions which may affect food flavor and appearance. The loss of Vitamin-C as well as the degradation of chlorophyll in vegetables can be a measure of improper storage temperature. Objective nutrition tests, quality assurance, and sensory analysis should be used to determine vegetable shelf life **(Singh, 1994)**.

The shelf life for frozen products is estimated by using models of shelf life kinetics **(Giannakourou and Taoukis, 2003)** or accelerated shelf life testing, consumer and sensory panels **(Martins and Silva, 2004)**.

2.2 Freezing process

Freezing of food starts when the food is placed in contact with a cold solid, or liquid or gaseous medium. For example, heat exchanger plates at -30 to -40˚C, solid carbon dioxide (dry ice) at -78.5˚C, liquid immersion in a cooling mixture or cryogenic fluid such as liquid nitrogen at -196˚C or gas (a stream of air, gaseous

nitrogen or CO2). The surface of the food cools faster than the centre of the food because the heat from the interior of the food has to reach the surface by conduction **(Evans, 2008)**.

Figure (2.1) shows a typical temperature record during freezing. The starting temperature T_0, temperature at the surface of the food may show (point A (t_1, T_s)) before increasing momentarily to approximately the initial freezing temperature T_f, and thereafter continuing along the 'thermal arrest' plateau (the B–C part) as transfer of the latent heat of freezing of water from the food begins. The first ice crystals are formed between A and B and further crystals are formed all the way to the final temperature T_e where the temperature of the food equilibrates to the temperature of the cooling medium **(Evans, 2008)**.

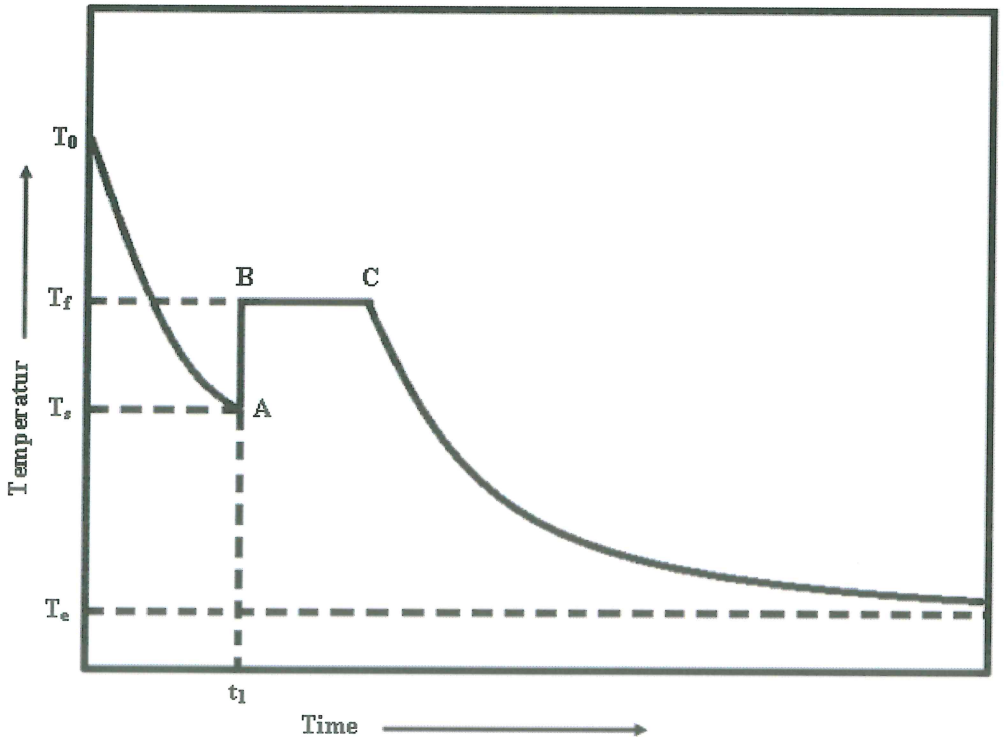

Figure (2.1): A schematic plot of temperatures in food during freezing.

Figure (2.2) shows the general freezing processing chart for vegetable products as described by **Mallett (1993)**.

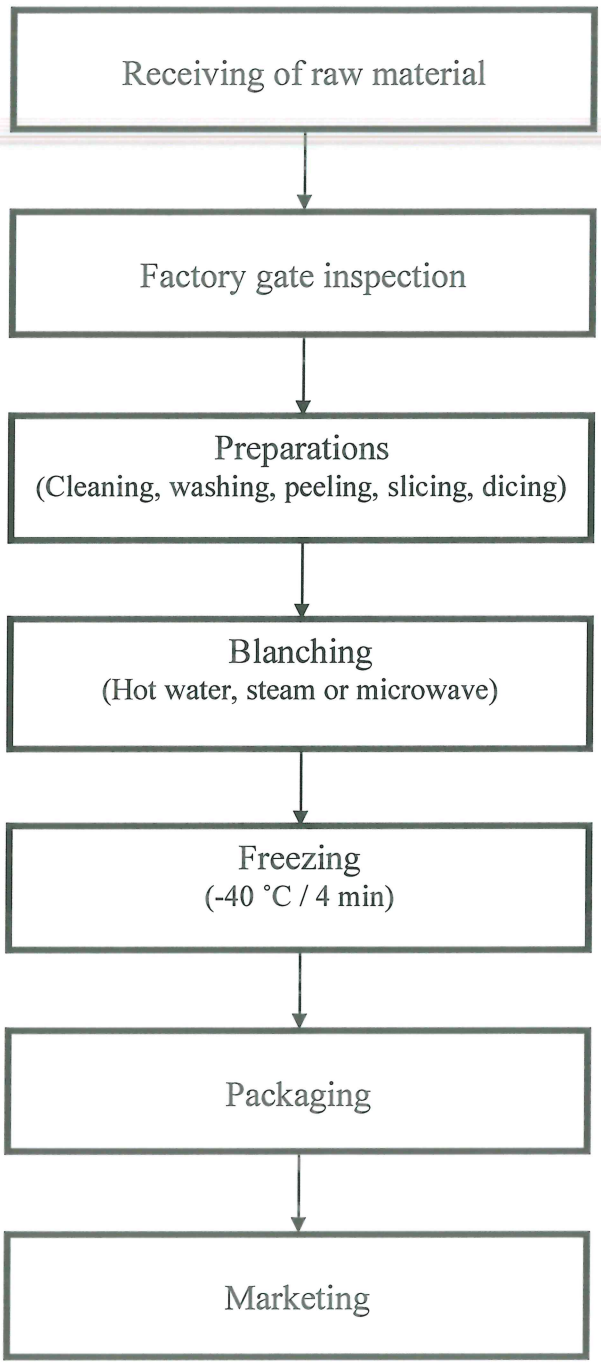

Figure (2.2): A general flow chart of frozen fruits and vegetables.

2.2.1 Freezing methods and equipments

In general, the industrial freezing methods and equipments that usually used during commercial production of frozen vegetables can be classified under the followings:

2.2.1.1 Air-blast freezers

The air blast freezer is considered as the oldest and commonly used freezing equipment due to its temperature stability and versatility for several food products. In this method, air is used as a freezing medium in the freezing device and freezing is accomplished by placing the food in freezing rooms called sharp freezers. In which the freezing time is largely dependent on the temperature and type of the freezing chamber, initial temperature, and size of product. The continuous freezers were found the most suitable system for mass production of packaged products with similar freezing times, in which the product is carried through on trucks or on conveyors. However, the batch freezers are more flexible since a variety of products can be frozen at the same time on individual trolleys. But the process requires closer supervision than the continuous system as the over-loading may be a great problem in these types of freezers **(Persson and Lohndal, 1993; Sipos *et al.*, 2009)**. An improved version of the air freezer is the forced air freezer, which consists of air circulation by conviction inside the freezing room **(Sipos *et al.*, 2009)**. A typical design for air blast freezer is shown in Figure (2.3).

2.2.1.2 Tunnel freezers

The fresh products on trays are placed in racks or trolleys inside a tunnel freezer. In order to be frozen by using cold air circulation. Optimum space is provided between layers of trolley, which can be moved continuously in and out of the freezer manually or by forklift trucks. This freezing system is suitable for all types of products, although there are some mechanical constraints including the requirement of high manpower for handling, cleaning, and transportation of trays

(Mallett, 1993; Fellows, 2000). A trolley for a tunnel freezer is shown in Figure (2.4).

2.2.1.3 Belt freezers

Belt freezers were designed to provide continuous frozen products flow by using a vertical air flow to force air through the product layer. The belts can be arranged in a multi-tier belt freezer or a spiral belt freezer. The spiral belt freezers consist of a belt that can be bent laterally around a rotating drum to maximize belt surface area in a given floor space **(Mallett, 1993; Sipos *et al.*, 2009)**. A typical spiral belt freezer is shown in Figure (2.5).

2.2.1.4 Fluidized bed freezers

The fluidized bed freezer consists of a bed with a perforated bottom through which cold air is blown vertically upwards. The system relies on forced cold air from beneath the conveyor belt, causing the products to suspend or float in the cold air stream **(George, 1993; Rahman, 1999)**. The use of high air velocity is very effective for freezing unpacked foods, especially when they can be completely surrounded by flowing air, as in the case of fluidized bed freezers.

The use of fluidization has several advantages compared with other methods of freezing since the product is individually quick frozen (IQF), which is convenient for particles with a tendency to stick together **(Fellows, 2000)**. Small vegetables are some of the products now frozen with this technology. A typical fluidized-bed freezer is shown in Figure (2.6).

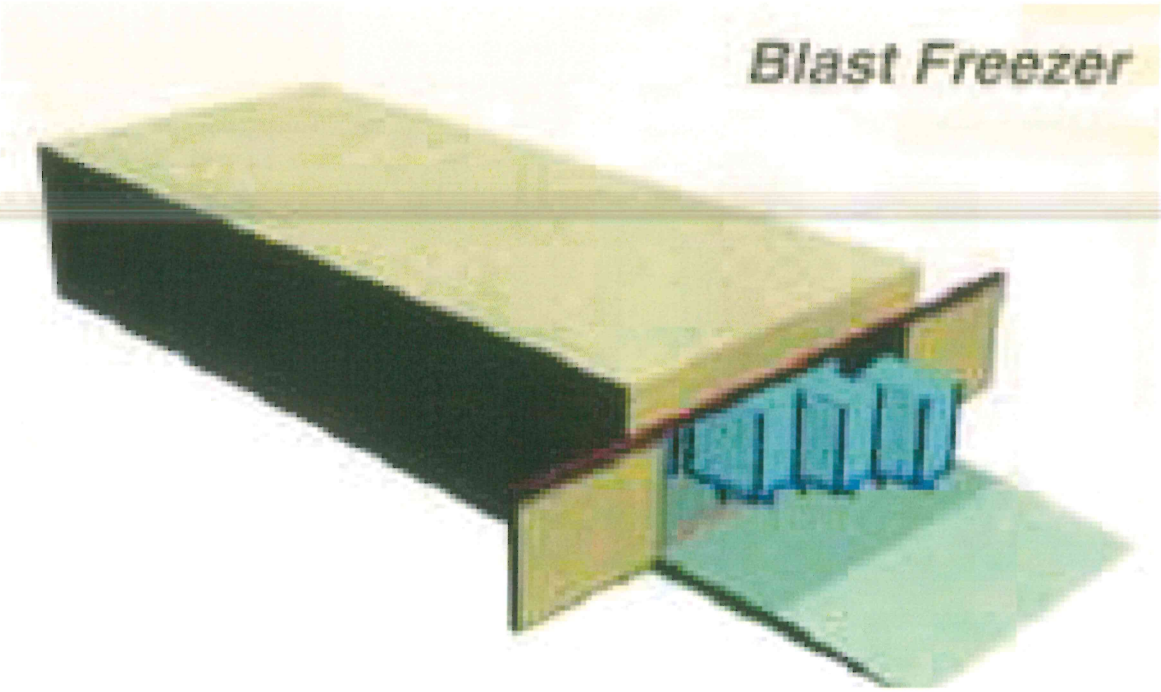

Figure (2.3): Air blast freezer

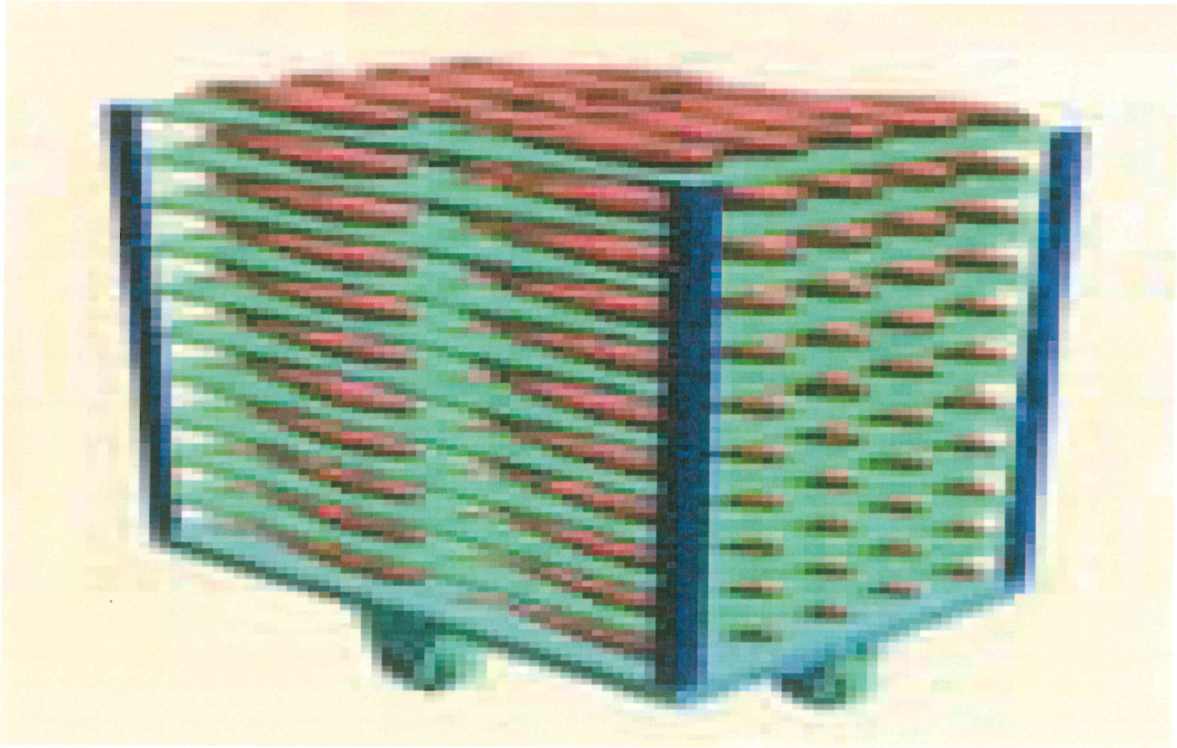

Figure (2.4): Trolley in a tunnel freezer

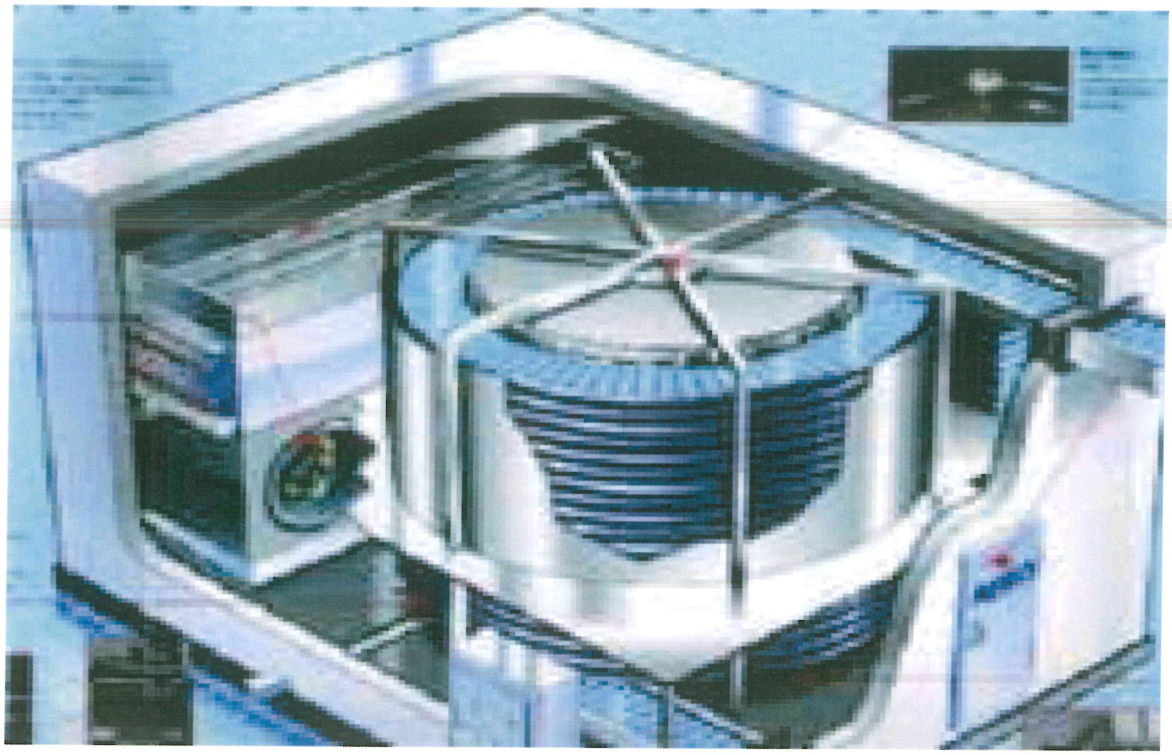

Figure (2.5): The cross-section view of a spiral belt freezer.

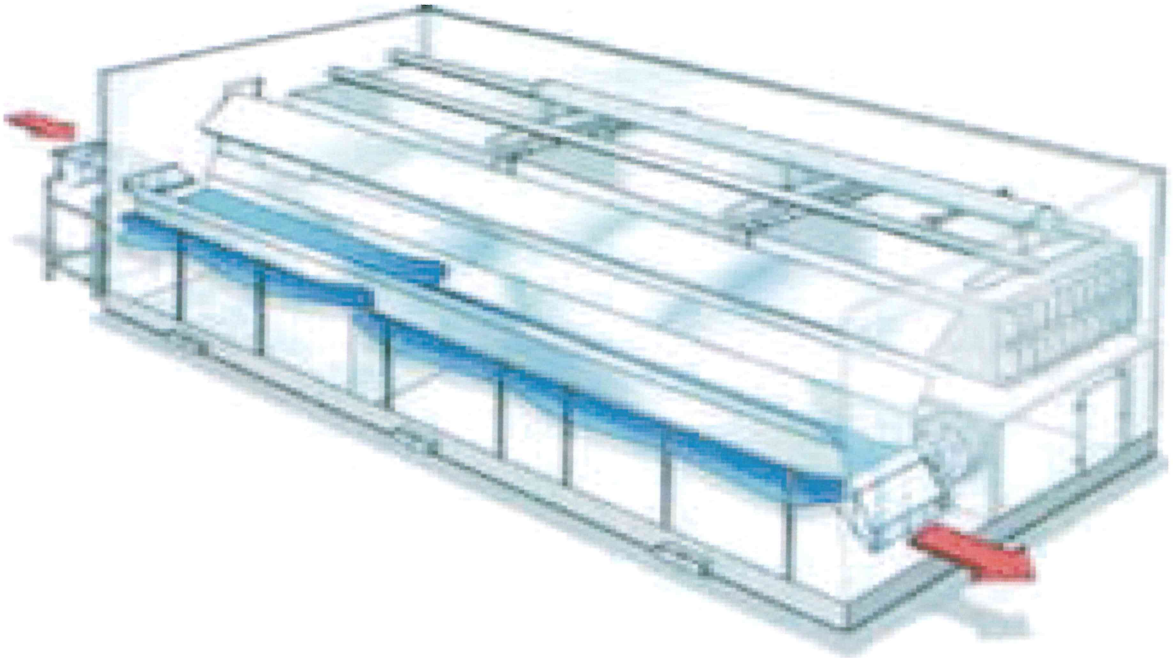

Figure (2.6): Cross-section view of a fluidized bed freezer.

2.2.1.5 Contact freezers

The contact freezing method is one of the most efficient ways of freezing in terms of heat transfer mechanism. In this method, the product could be in direct or indirect contact with the freezing medium. For direct contact freezers, the product being frozen is fully surrounded by the freezing medium, the refrigerant, maximizing the heat transfer efficiency **(Mallett, 1993; Fellows, 2000)**. A schematic illustration is given in Figure (2.7).

2.2.1.6 Indirect contact freezers

The mechanism of indirect contact freezer is shown in Figure (2.8). However, indirect contact freezers were mentioned to provide an efficient medium for heat transfer **(Fellows, 2000)**.

2.2.1.7 Immersion freezers

The immersion freezer consists of a tank with a cooled freezing media, such as glycol, glycerol, sodium chloride, calcium chloride, or mixtures of salt and sugar. The product is immersed in this solution or sprayed while being conveyed through the freezer, resulting in fast temperature reduction through direct heat exchange **(Hung and Kim, 1996)**. Direct immersion of a product into a liquid refrigerant is the most rapid way of freezing since liquids have better heat transfer properties than air. The solute used in the freezing system should be safe without taste, odour, colour, or flavour, and for successful freezing, products should be greater in density than the solution. Immersion freezing systems have been commonly used for shell freezing of large particles due to the reducing ability of product dehydration when the outer layer is frozen quickly **(George, 1993; Sipos *et al.*, 2009)**. A simple illustration of the immersion freezer is shown in Figure (2.9).

2.2.1.8 Contact belt freezers

This type of freezer is designed with single-band or double-band for freezing of thin product layers as shown in Figure (2.10). The design can be either straight forward or drum **(Persson and Lohndal, 1993; Fellows, 2000)**.

2.2.1.9 Plate freezers

The most common type of contact freezer is the plate freezer, in which the product is pressed between hallow metal plates, either horizontally or vertically, with a refrigerant circulating inside the plates. Pressure is applied for good contact as schematically shown in Figure (2.11a). This type of freezing system is only limited to regular-shaped materials or blocks or block-shaped packaged products. A typical plate freezer is shown in Figure (2.11b) as reported by **Fellows (2000)**.

2.2.1.10 Cryogenic freezers

Cryogenic freezing is a relatively new freezing method in which the food is exposed to an atmosphere below -60 °C through direct contact with liquefied gases such as nitrogen or carbon dioxide. This type of system differs from other freezing systems since it is not connected to a refrigeration plant; the refrigerants used are liquefied in large industrial installations and shipped to the food-freezing factory in pressure vessels. Thus, the small size and mobility of cryogenic freezers allow for flexibility in design and efficiency of the freezing application. Low initial investment and high operating costs are typical constrains for cryogenic freezers **(Hung and Kim, 1996; Fellows, 2000)**.

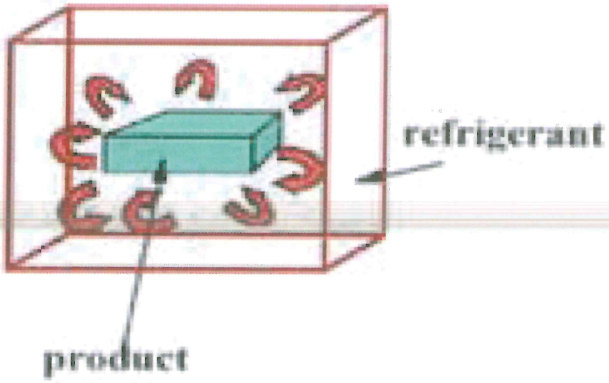

Figure (2.7): Direct contact freezer

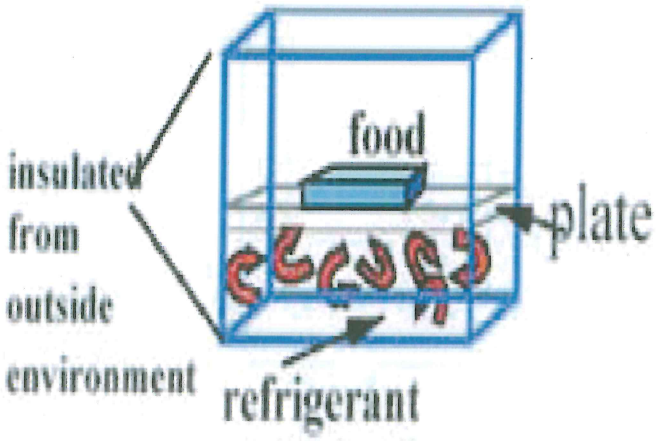

Figure (2.8): Indirect contact freezer

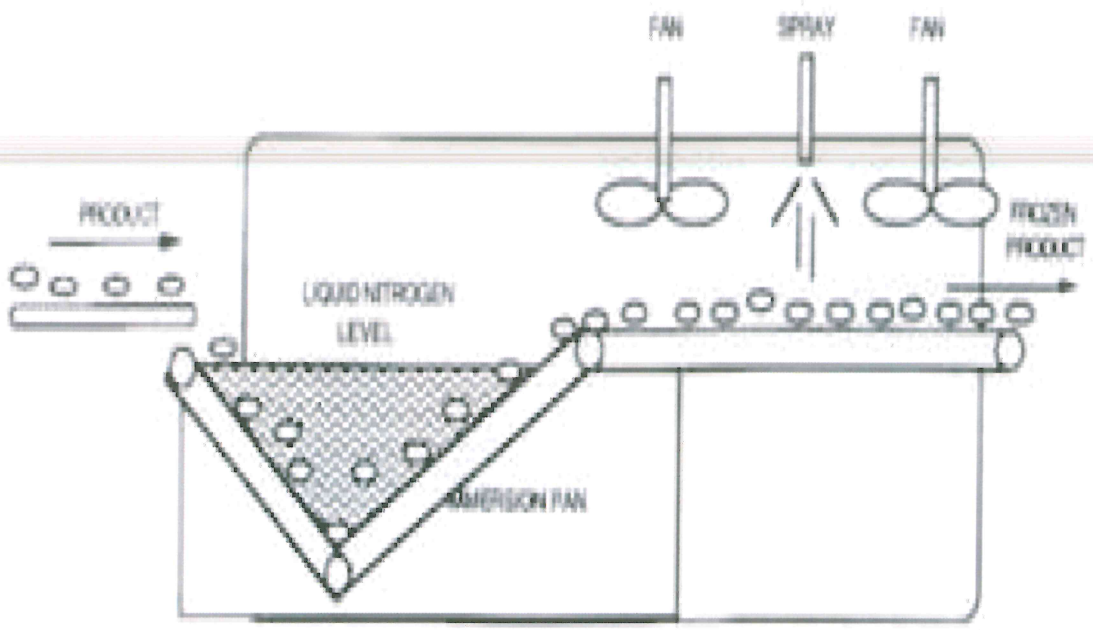

Figure (2.9): Immersion freezer

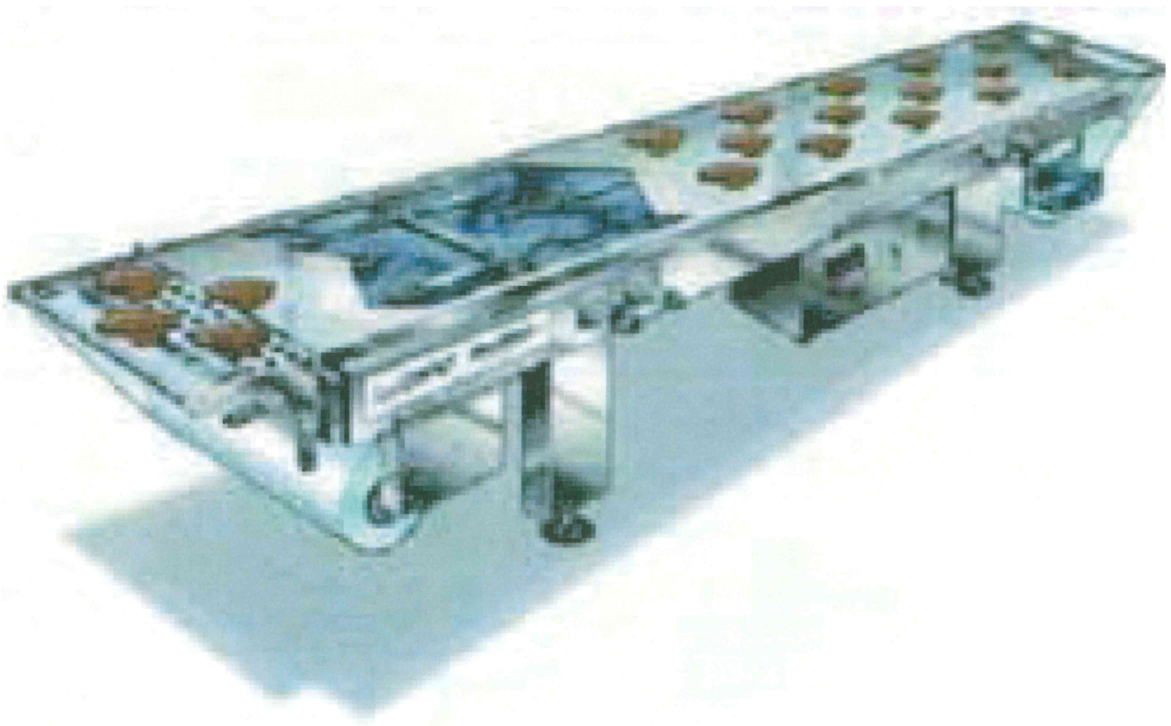

Figure (2.10): Contact belt freezer

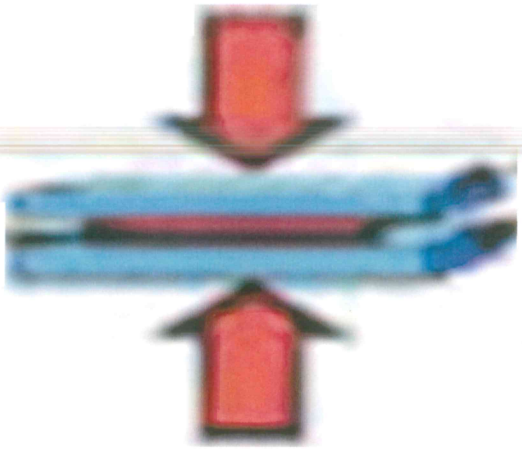

Figure (2.11a): Pressure application in a plate freezer.

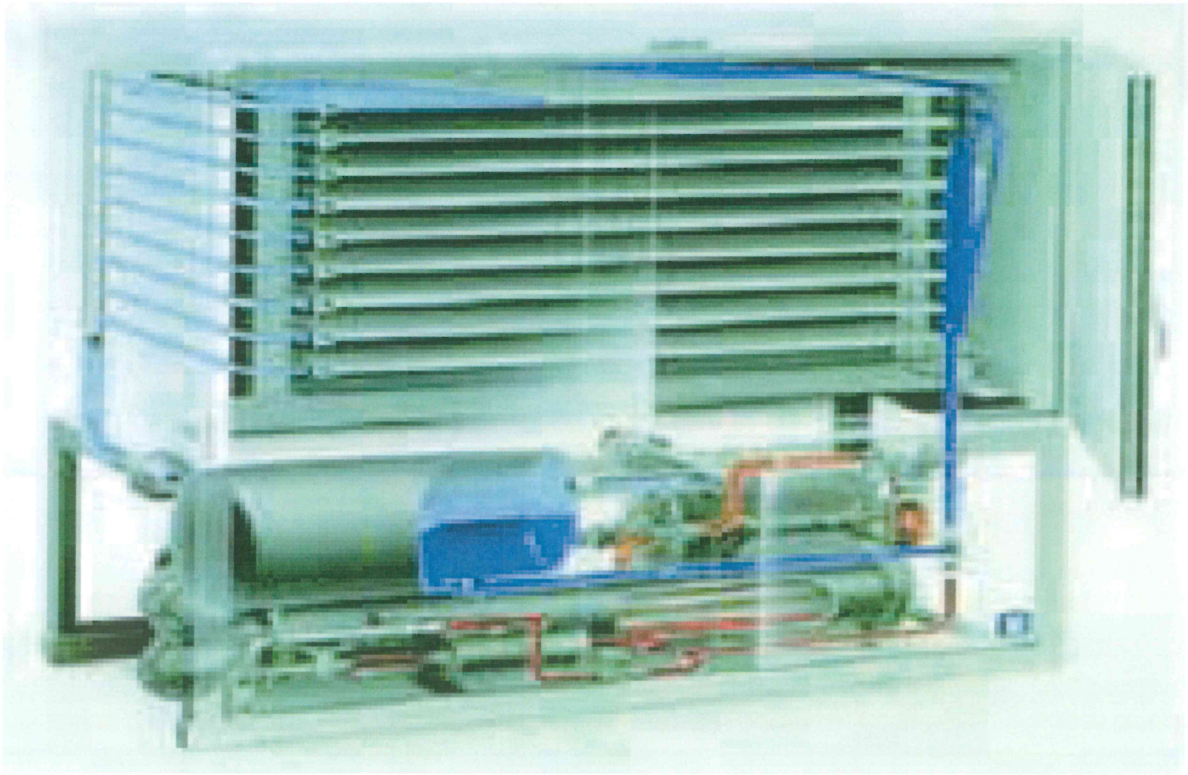

Figure (2.11b): Plate freezer with a two-stage compressor and sea water condenser.

2.2.2 Packaging of frozen vegetables

Arthey (1993) stated that, there are several factors should be considered in packaging frozen vegetables. These include protecting food from atmospheric oxygen, prevention of moisture loss, retention of flavour, and increasing the rate of heat transfer through the package. As reported by **Harrison and Croucher (1993)**, there are three types of packaging used for frozen foods: primary, secondary, and tertiary. The primary package is in direct contact with food and the food is kept inside it up to the time of utilization. The secondary packaging is a form of multiple packaging used to handle packages together for sale. While, the packaging is used for bulk transportation of products. In general, the containers should be leakage free while easy to seal. Durability of the material is another important factor to consider, since the packaging material must not become brittle at low temperatures and crack.

The packaging materials should be moisture-vapor-proof to prevent evaporation, thus retaining the highest quality of frozen foods. Oxygen should also be completely evacuated from the package using a vacuum or gas-flush system to prevent migration of moisture and oxygen **(Sebranek, 1996)**. The package of frozen vegetables should be attractive to the consumer, protected from external contamination, and effective in terms of processing, handling, and cost **(Rahman, 1999)**.

A range of different packaging materials, grouped as rigid and non-rigid containers can be used for primary packaging. Glass, plastic, tin, and heavily waxed cardboard materials are considered as rigid containers and are usually used for packaging of liquid food products. Glass containers are mostly used for vegetables if they are not water-packed. Non-rigid containers include bags and sheets made of moisture-vapor-resistant heavy aluminum foil, polyethylene or laminated papers. Bags are the most commonly used packaging materials for frozen vegetables due to their flexibility during processing and handling. They can

be used with or without outer cardboard cartons to protect against tearing **(Barbosa-Cánovas *et al.*, 2005)**. As mentioned by **Sipos *et al.*, (2009)**, proper packaging of frozen food is important to protect the product from contamination and damage during transportation, as well as to preserve food value, flavour, colour, and texture.

According to **Garden-Robinson (2013)**, there are two basic packing methods that recommended for frozen vegetables. The first method is the dry pack method, in which the blanched and drained vegetables are put into meal-sized freezer bags and packed tightly. Proper headspace (approximately 2 cm) is left at the top of rigid containers before closing. Provision for headspace is not necessary for foods such as broccoli, asparagus, and brussels sprouts, as they do not pack tightly in containers. While, the second method is the tray pack method, in which chilled, well-drained vegetables are placed in a single layer on shallow trays or pans. Trays are placed in a freezer until the vegetables become firm. Then the vegetables are removed and filled into containers. The tray-packed foods do not freeze in a block but remain loosely distributed so that the amount needed can be poured from the container and the package reclosed.

2.2.3 Quality and specifications of frozen vegetable products

2.2.3.1 Quality and consumer satisfaction

Cardello (1995) and Becker (2000), showed that the concept of "quality" is comparable to "fresh" which is also a multidimensional word and was highly dependent on the individual. The experimental influence on the concept of freshness was tested by **Cardello and Schutz (2003)**. It is likely that, individual characteristics can similarly influence the perception of freshness **(Ragaert *et al.*, 2004)**.

2.2.3.2 Specifications of frozen vegetable products

2.2.3.2.1 Specification of frozen okra

Product Description: Frozen Okra, graded, sorted, washed, blanched, frozen.

Table (2.6) shows the general specifications of frozen green okra as stated by Egyptian Standard No. 1702 / 2005.

2.2.3.2.2 Specification of frozen green beans

Product Description: Frozen Green Beans, washed, blanched, frozen.

Table (2.7) indicates the general specifications of frozen green beans as reported by the Codex and Egyptian Standard No. 113 / 1981 and 1743 / 2005, respectively.

2.2.3.2.3 Specification of frozen peas

Product Description: Frozen Peas, washed, blanched, frozen.

Table (2.8) stated the general specifications of frozen green peas as mentioned by the Codex and Egyptian Standard No. 41 / 1981 and 1748 / 2005, respectively.

2.2.3.2.4 Specification of frozen spinach

Product Description: Frozen Spinach, washed, blanched, frozen.

Table (2.9) illustrates the general specifications of frozen spinach as indicated by the Codex and Egyptian Standard No. 771 / 1981 and 1749 / 2005, respectively.

2.2.3.2.5 Specification of frozen molokhia

Product Description: Frozen Molokhia, washed, blanched, frozen.

Table (2.10) shows the general specifications of frozen molokhia as described by Egyptian Standard No. 1681 / 2005.

Table (2.6): Specifications of frozen green okra

Appearance	Frozen green okra were graded, cleaned, sorted, blanched, frozen, and packed in suitable package for frozen storage.
Colour	Uniform Green
Smell and Flavour	Typical, fresh and free from strange taste and smell.
Consistency	Soft, not Fibrous, not tough
Form / Size	Extra ≤ 2cm Tolerance 5% (numerically) Zero >2cm - <3cm Tolerance 5% (numerically) Premium >3cm - <4cm Tolerance 5% (numerically) Green Okra >4cm - <5cm Tolerance 5% (numerically)
Foreign material	Absent
Foreign vegetable	Absent
Blemished	Not exceed 1 Piece
Mechanical damage	Not exceed 1 % weight / weight
Steams	Absent
Yellowish/browning	5 % by weight
Insect/Fungus injury	Absent
Freezing burn	Absent
Seeds discrete	Absent
Storage & marketing	-18°C
Packaging Details	Product name, weight, production date, expiry date, Storage temperature, production code, company details, Origin. Do not refreeze after defrosting.

Microbial Test	**Standard Range**	**Used Method**
Total Plate Count	< 100,000 colony/g	ISO 4833/2003 3rd. Edition
Coliforms	< 100 colony/g	ISO 4832-2006 3rd. Edition
E-Coli	Absent	ISO 4832-2006 3rd. Edition
Yeast	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Mould	< 50 colony/g	ISO 21527-2/2008 1ST. Edition

Chemical Test	**Standard Range**	**Used Method**
Pesticides residues	< 0.01 mg/kg	European Standard 15662 / 2008
Heavy metals	< 0.01 mg/kg	European Standard 1881/2006
Oxidative enzymes	Negative	Egyptian Standard 991 / 2008

Table (2.7): Specifications of frozen green beans

Appearance	Green Beans were cleaned, cut, ends removed, sorted, blanched, frozen, and packed in suitable package for frozen storage.	
Colour	Even Green	
Smell and Flavour	Typical, fresh and free from strange taste and smell.	
Texture	Not tough	
Length	< 1 cm maximum 2 % by weight > 3 cm maximum 2 % by weight	
Foreign material	Absent	
Foreign vegetable	Absent	
Rusty spots, damage	Not exceed 1 Piece	
Ends not removed	Not exceed 1 % by weight	
Oxidized ends	Maximum 1 Piece	
Yellowish/browning	1 % by weight	
Insect/Fungus injury	Absent	
Freezing burn	Absent	
Seeds discrete	Absent	
Storage & marketing	-18°C	
Packaging Details	Product name, weight, production date, expiry date, Storage temperature, production code, company details, Origin. Do not refreeze after defrosting.	
Microbial Test	**Standard Range**	**Used Method**
Total Plate Count	< 100,000 colony/g	ISO 4833/2003 3rd. Edition
Coliforms	< 100 colony/g	ISO 4832-2006 3rd. Edition
E-Coli	Absent	ISO 4832-2006 3rd. Edition
Yeast	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Mould	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Chemical Test	**Standard Range**	**Used Method**
Pesticides residues	< 0.01 mg/kg	European Standard 15662 / 2008
Heavy metals	< 0.01 mg/kg	European Standard 1881/2006
Oxidative enzymes	Negative	Egyptian Standard 991 / 2008

Table (2.8): Specifications of frozen green peas

Appearance	Green peas were cleaned, sorted, blanched, frozen, and packed in suitable package for frozen storage.	
Colour	Uniform Green	
Smell and Flavour	Typical, fresh and free from strange taste and smell.	
Texture	Soft, consistent	
Size (Diameter)	6 – 8 mm	
Foreign material	Absent	
Foreign vegetable	Maximum 5 % weight / weight Not Exceed 12Cm^2	
Broken	< 6 mm maximum 5 % weight / weight	
Mechanical damage	Absent	
Yellowish/browning	2 % weight / weight	
Insect/Fungus injury	Absent	
Freezing burn	Absent	
Storage & marketing	-18°C	
Packaging Details	Product name, weight, production date, expiry date, Storage temperature, production code, company details, Origin. Do not refreeze after defrosting.	
Microbial Test	**Standard Range**	**Used Method**
Total Plate Count	< 100,000 colony/g	ISO 4833/2003 3rd. Edition
Coliforms	< 100 colony/g	ISO 4832-2006 3rd. Edition
E-Coli	Absent	ISO 4832-2006 3rd. Edition
Yeast	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Mould	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Chemical Test	**Standard Range**	**Used Method**
Pesticides residues	< 0.01 mg/kg	European Standard 15662 / 2008
Heavy metals	< 0.01 mg/kg	European Standard 1881/2006
Oxidative enzymes	Negative	Egyptian Standard 991 / 2008
Alcohol–Insoluble Solid content	23%weight/weight	AOAC 971.29
Flotation (Maturity)	59% Floats in salt solution	Egyptian Standard 1748 / 2005

Table (2.9): Specifications of frozen spinach

Appearance	Spinach were fully growth, cleaned, sorted, blanched, chopped, frozen, and packed in suitable package for frozen storage.	
Colour	Uniform Green	
Smell and Flavour	Typical, fresh and free from strange taste and smell.	
Consistency	Homogeneous Sticky	
Strange Grasses	Absent	
Foreign material	Absent	
Foreign vegetable	Absent	
Yellowish patches	Absent	
Black Patches	Absent	
Storage & marketing	-18°C	
Packaging Details	Product name, weight, production date, expiry date, Storage temperature, production code, company details, Origin. Do not refreeze after defrosting.	
Microbial Test	**Standard Range**	**Used Method**
Total Plate Count	< 100,000 colony/g	ISO 4833/2003 3rd. Edition
Coliforms	< 100 colony/g	ISO 4832-2006 3rd. Edition
E-Coli	Absent	ISO 4832-2006 3rd. Edition
Yeast	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Mould	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Chemical Test	**Standard Range**	**Used Method**
Pesticides residues	< 0.01 mg/kg	European Standard 15662 / 2008
Heavy metals	< 0.01 mg/kg	European Standard 1881/2006
Oxidative enzymes	Negative	Egyptian Standard 991 / 2008
Moisture	95%	Egyptian Standard 991 / 2008
Ash (insoluble in acid)	0.1%	Egyptian Standard 991 / 2008
Zn	< 0.1mg/kg	Egyptian Standard 2360 / 2007
Cm	< 0.1mg/kg	Egyptian Standard 2360 / 2007
Pb	< 0.2mg/kg	Egyptian Standard 2360 / 2007

Table (2.10): Specifications of frozen molokhia

Appearance	Molokhia were cleaned, sorted, blanched, chopped, frozen, and packed in suitable package for frozen storage.	
Colour	Uniform Green	
Smell and Flavour	Typical, fresh and free from strange taste and smell.	
Consistency	Sticky	
Strange Grasses	Absent	
Foreign material	Absent	
Foreign vegetable	Absent	
Yellowish patches	Absent	
Black Patches	Absent	
Storage & marketing	-18°C	
Packaging Details	Product name, weight, production date, expiry date, Storage temperature, production code, company details, Origin. Do not refreeze after defrosting.	
Microbial Test	**Standard Range**	**Used Method**
Total Plate Count	< 100,000 colony/g	ISO 4833/2003 3rd. Edition
Coliforms	< 100 colony/g	ISO 4832-2006 3rd. Edition
E-Coli	Absent	ISO 4832-2006 3rd. Edition
Yeast	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Mould	< 50 colony/g	ISO 21527-2/2008 1ST. Edition
Chemical Test	**Standard Range**	**Used Method**
Pesticides residues	< 0.01 mg/kg	European Standard 15662 / 2008
Heavy metals	< 0.01 mg/kg	European Standard 1881/2006
Oxidative enzymes	Negative	Egyptian Standard 991 / 2008
Moisture	94.5%	Egyptian Standard 991 / 2008
Ash	1.5%	Egyptian Standard 991 / 2008
Ash (insoluble in acid)	0.15%	Egyptian Standard 991 / 2008

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Materials

The present study was carried out in Upper Egypt Factory for Food Industries (about 120km south Cairo city). The fresh vegetables samples namely Okra (*Abelmoschus esculentus*), Green Beans (*Phaseolus vulgaris*), Peas (*Pisum sativum*), Spinach (*Spinacia oleracea*) and Molokhia (Jews mallow) (*Corchorus olitorius*) were collected at the factory gate and washed under running tap water and kept at 4 ˚C until needed for the chemical investigations. Also, another vegetables samples were collected after blanching, freezing and during frozen storage period (12 months). The different samples were kept at -18 °C until needed for the different investigations. All the chemicals used in this study are of analytical grades which were donated by the aforementioned factory.

3.2 Methods

3.2.1 Vegetables freezing methods

Fresh vegetables namely okra, green beans, peas, spinach and molokhia were cleaned, washed, blanched at (95 – 100 °C), freezed at (-35) – (-40 °C) and then stored at -18 °C for 12 months. Figures (3.1), (3.2), (3.3), (3.4) and (3.5) show the freezing processing chart for okra, green beans, peas, spinach and molokhia, respectively.

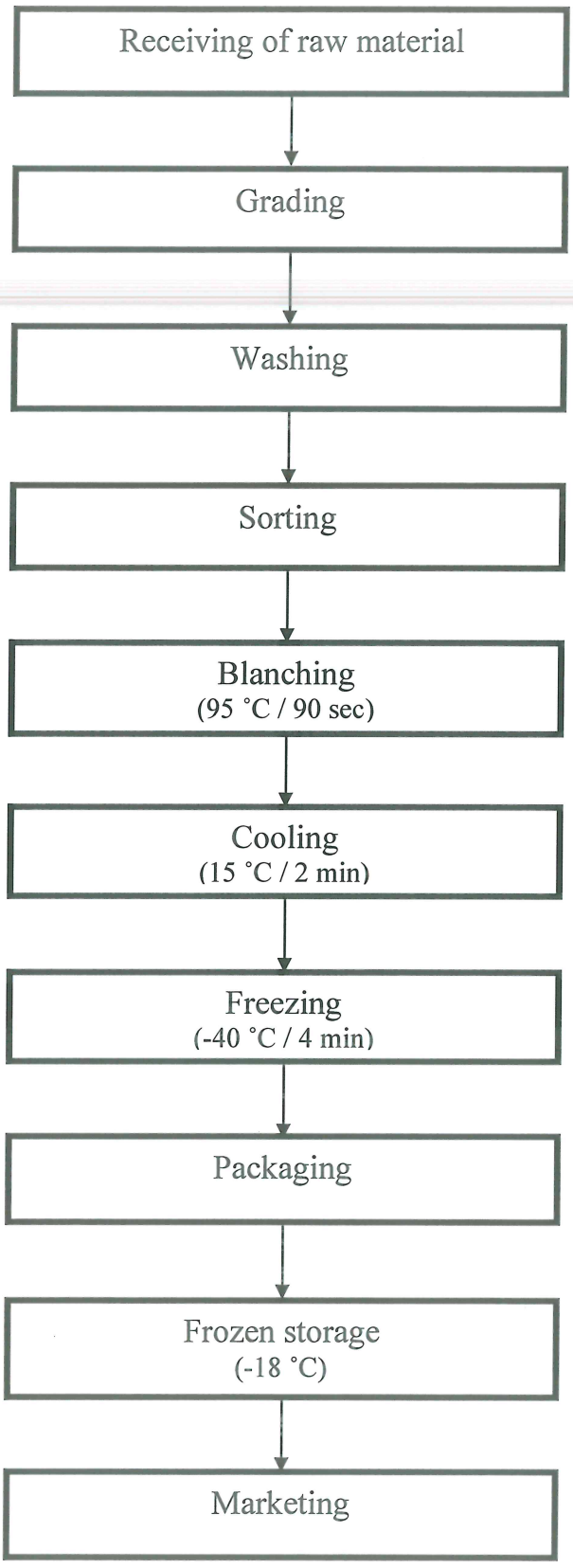

Figure (3.1): Flow chart of okra freezing method

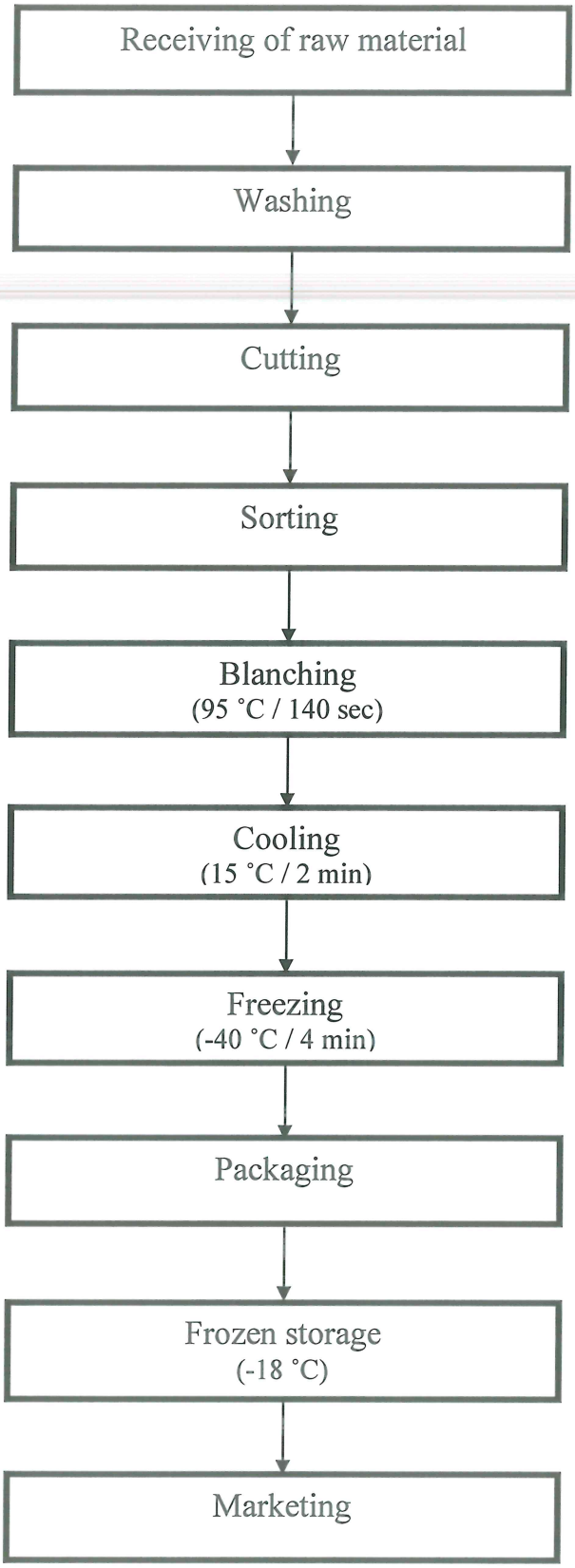

Figure (3.2): Flow chart of green beans freezing method

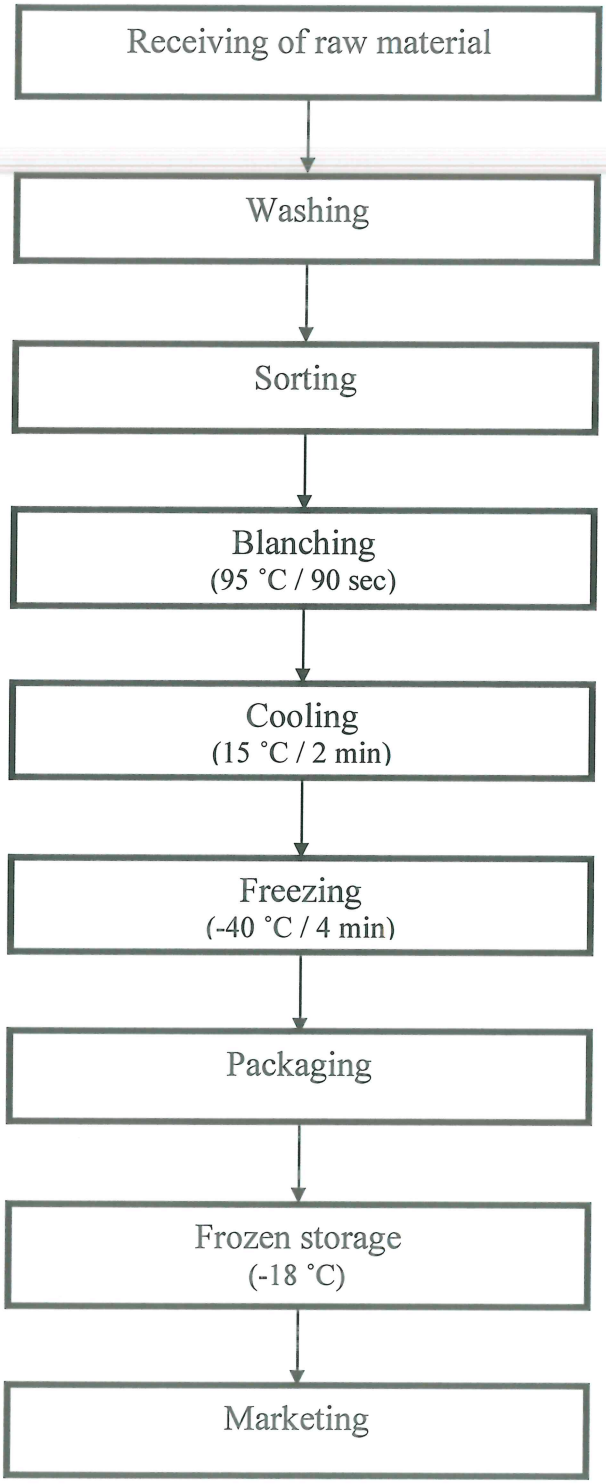

Figure (3.3): Flow chart of peas freezing method

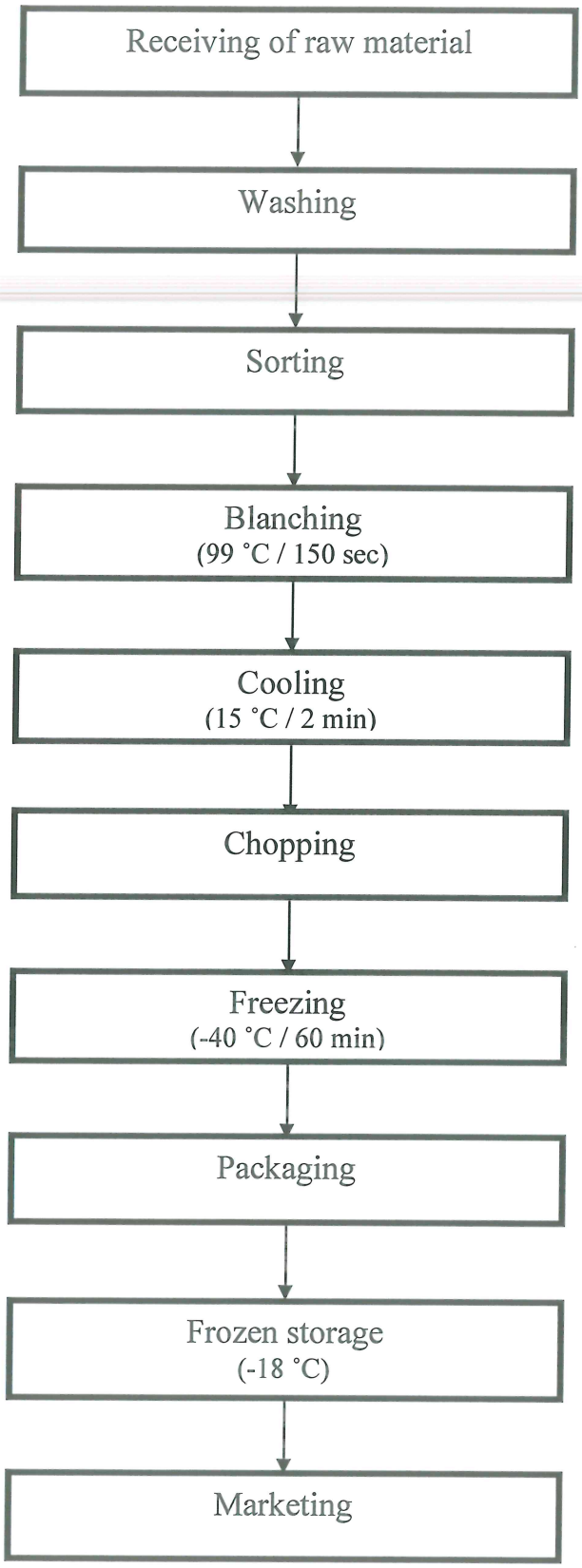

Figure (3.4): Flow chart of spinach freezing method

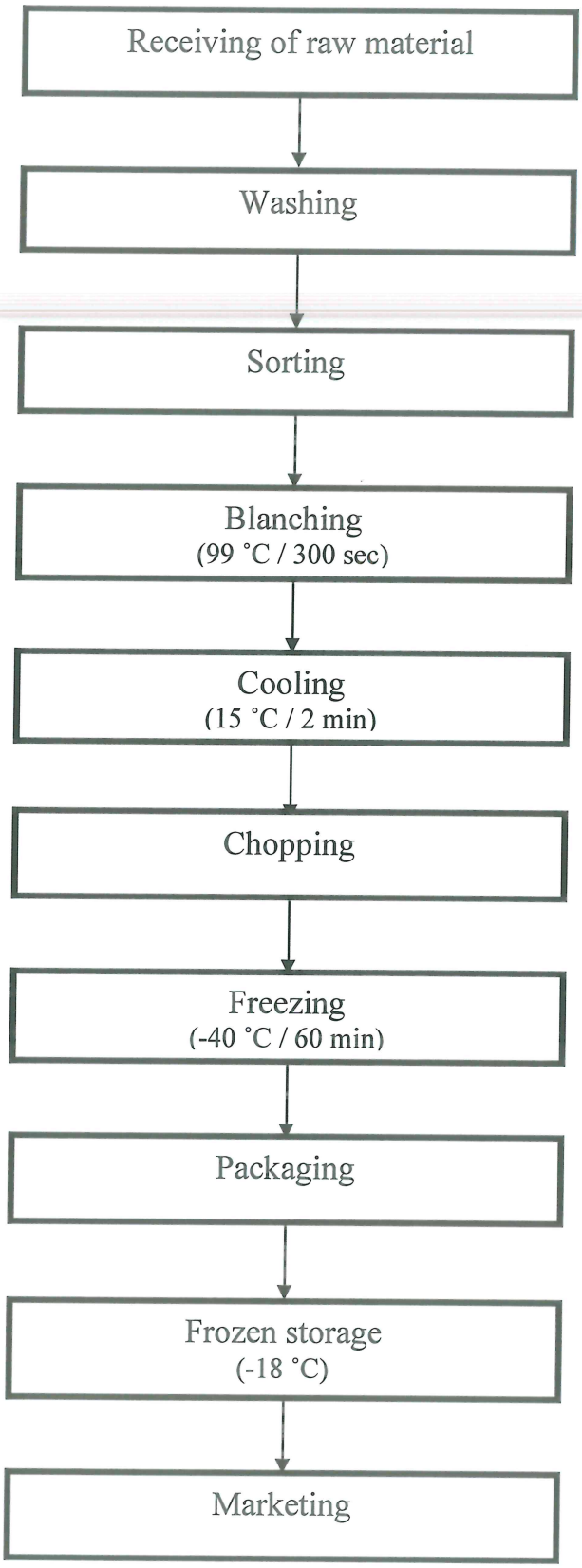

Figure (3.5): Flow chart of molokhia freezing method

3.2.2 Chemical methods

3.2.2.1 Moisture content

The moisture content in each sample was determined following the standard method described by the **AOAC (2010)**.

Five grams of a well mixed sample was weighed accurately in clean and dried Petri dishes by using a sensitive balance (Model No. AR2140, OHAC, SCORO, USA). Then, the Petri dishes with the samples were placed in an oven (Carblite, Sheffield, England) at 105°C for five hours. After that the Petri dishes were transferred to a desiccator and re-weighing after cooling to room temperature. Again, the Petri dishes were transferred to the oven and weighed after two hours and this repeated till a constant weight was obtained. Then, the moisture content (%) was calculated as the loss in weight after drying:

$$\text{Moisture content (\%)} = \frac{(w_s - w_d) \times 100\ \%}{\text{sample weight(g)}}$$

Where:

w_s = weight of sample before drying (g).

w_d = weight of sample after drying (g).

3.2.2.2 Protein content

The protein content in the different samples was determined by using the micro- Kjeldahl method according to the **AOAC (2010).**

Principle:

The sample was digested with a strong sulphuric acid. The sample releases its nitrogen content which can be determined by a suitable titration technique. A conversion factor of 6.25 (equivalent to 16g nitrogen per 100 grams of protein) was used in this method to calculate the sample protein content. The method is divided into three steps which can be summarized under the followings:

Digestion

A sample of ten grams (10 ± 1mg) was transferred into a digestion flask and then digested by heating for 2-3 hours in (3.5N) sulphuric acid. The digestion process was catalyzed by a mixture 0.4 of 10 parts K_2SO_4 to one part of $CuSO_4$. The heating was continued till the black colour turned to pale blue and the fumes disappeared which indicate that the digestion process was completed.

Distillation

After the digestion has been completed the digestion flask was cooled and transferred to distillation unit using a minimum volume of water. The solution in the distillation unit was then turned alkaline by addition of 20 ml of sodium hydroxide (40%) to release the ammonia. Then, the released ammonia was distilled into 20ml of 2% boric acid in conical flask with 2 to 3 drops of bromochresol methyl red as indicator.

Titration

The nitrogen content in the sample was then estimated by titration of the ammonium borate formed with a standard hydrochloric acid (0.1N). The titrations continued till the colour of the solution turned to red (pink). Then, the following formula was used to determine the protein content as %:

$$\text{Protein content (\%)} = \frac{N \times T \times 14 \times 6.25 \times 100\%}{1000 \times \text{sample weight (g)}}$$

Where:

N = Normality of HCl.

T = Actual volume of HCl used for sample titration (ml HCl – ml blanck).

1000 = Number of milligrams in one gram.

14 = Equivalent weight of nitrogen.

6.25 = Protein conversion factor.

3.2.2.3 Fat content

The Fat content in the sample was determined by using continuous extraction apparatus (Soxhelt type), as described by the **AOAC (2010)**.

Five grams (5 ± 1mg) samples were weighed and transferred to an extraction thimble covered with a piece of glass wool and then placed in the Soxhlet apparatus. After that, the solvent (petroleum ether) was added into a deried weighed Soxhlet flask and the extraction process was continued for about six hours. Then, the samples were dried in an oven (Catblite, Sheffield, England) for 30 minutes to eliminate any remaining amounts of the solvent and the flask was reweighted. The fat content as % was calculated by using the following equation:

$$\text{Fat content (\%)} = \frac{(w_2 - w_1) \times 100\ \%}{\text{sample weight(g)}}$$

Where:

w_1 = weight of the empty Soxhelt flask (g).

w_2 = weight of Soxhelt flask with fat content (g).

3.2.2.4 Total carbohydrates

The total carbohydrates as % were calculated by subtracting the sum of moisture, protein, fat and ash as per-cent from 100% as described by **West *et al.* (1988)**.

Total carbohydrates (%) = 100% – (moisture% + protein% + fat% + ash%)

3.2.2.5 Fiber content

The fiber content in each sample was determined according to the **AOAC (2010)**. About two grams sample were weighed and 200 ml sulphuric acid (0.26N) was added, boiled for 30 minutes and then filtered. The residue was washed three times by using hot water and after that 200 ml sodium hydroxide (0.26N) was added, boiled again for 30 minutes and filtered. Then, the residue was carefully washed three times with hot water until it was free from alkali. After that, the

samples were transferred to an oven (Carblite, Sheffield, England) at 105°C (overnight) and reweighed. The residue was ashed in a muffle furnace (LEF-103S, watts: 2KW10A, Korea) at 550 °C for three hours till a light gray ash was formed and a constant weight was obtained. The fiber content as % was calculated by using the following equation:

$$\text{Fiber content (\%)} = \frac{(w_1 - w_2) \times 100\ \%}{\text{sample weight(g)}}$$

Where:

w_1 = weight of sample before ignition (g).

w_2 = weight of sample after ignition (g).

3.2.2.6 Available carbohydrates

The available carbohydrates were calculated by subtracting the fiber percent from total carbohydrates percent as described by **West *et al.* (1988)**.

Available carbohydrates (%) = total carbohydrates% - fiber%

3.2.2.7 Ash content

The ash content in the different samples was determined according to the **AOAC (2010)**.

The empty crucibles were accurately weighed and then two grams sample were transferred to each crucible by using a sensitive balance (Model No. AR2140, OHAC, SCORO, USA). Then, the crucibles and the samples were placed in a muffle furnace (LEF-103S, watts: 2KW10A, Korea) at 550 to 700 °C for more than 6 hours until a white to gray ash was obtained. After that, the crucibles were transferred from the furnace to a desiccator to cool to room temperature and re-weighed. The ash content as % was calculated by using the following equation:

$$\text{Ash content (\%)} = \frac{(w_1 - w_2) \times 100\ \%}{\text{sample weight(g)}}$$

Where:

w_1 = weight of crucible with the remaining ash sample (g).

w_2 = weight of the empty crucible (g).

3.2.2.8 Ascorbic acid content

The ascorbic acid content was determined according to the modified **Ruck (1963)** method that described by **El-obeid (2003)**.

A sample of 30 ±1g was blended with about 100 ml of 0.4% oxalic acid (4grams/100ml) for 2 minutes in an electric blender. The blended mixture was made up to 500 ml in a volumetric flask with 0.4% oxalic acid and filtered. The ascorbic acid in the filtrate was determined by titrating 20ml of the filtrate against 2,6-dichlorophenol indophenols pigment (0.2g/500 ml distilled water) of known strength. The ascorbic acid was calculated and expressed as mg/100g dry matter according to the following equation:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre (ml)} \times \text{dye strength} \times 100}{\text{factor}}$$

Where:

$$\text{factor} = \frac{\text{Sample weigh} \times \text{sample volume taken for titration}}{\text{total volume of the sample}}$$

$$\text{Dye strength} = \frac{1}{\text{Titre}}$$

3.2.2.9 Minerals content

The minerals content in each sample was extracted according to **Pearson's (1981)** method.

The sample was burnt in a muffle furnace (LEF-103S, watts: 2KW10A, Korea) at 550 °C and placed in a sand bath for 10 minutes after addition of 10ml of 5N HCl. Then, the solution was carefully filtered in a 100 ml volumetric flask and made up to the mark distilled water. Sodium and potassium were determined by using Flame photometer (JENWAY 3110, UK) according to the **AOAC (2010)**. Calcium and magnesium were determined as described by **Chapmam and Pratt (1968) and Pearson's (1981)**, respectively.

3.2.2.10 Energy value

The energy values of the fresh and frozen vegetable products were calculated based on Atwater factors for protein, fat and available carbohydrates as indicated by **Leung (1968)**:

Protein factor = 3.87 (kcal/g)

Fat factor = 8.37 (kcal/g)

Carbohydrate factor = 4.12 (kcal/g)

1.0 kcal = 4.184 (kj)

3.2.3 Physico-chemical methods

3.2.3.1 Hydrogen ions concentration (pH)

The hydrogen ions concentration (pH) in the different samples was determined according to the **AOAC (2010)**.

Principle:

The pH value of each sample is measured potentiometrically with a pH-meter. After standardization of the meter electrode with buffer solutions, the reading is taken when the equilibrium potential across the electrode is achieved.

Procedure:

After standardization of the pH-meter (JENWAY, 3510 pH meter) with two buffer solutions (pH of 4.00 and 7.00), the electrode of the pH-meter was rinsed with distilled water, immersed in the sample solution (20˚C) and left to stand until a staple reading was achieved. All the readings were expressed as pH to the nearest 0.01 pH units.

3.2.4 Photometric methods

3.2.4.1 Determination of enzyme activity

The method that described by **Angela *et al.* (2001)** was used to prepare the crude enzymes extract. In this method, leaf tissues were homogenized in a chilled

pistel with 100 ml potassium phosphate buffer (pH 7.5) containing 1ml ethylene diamine tetra-acetic acid (EDTA), 3ml DL-dithiothreitol and 5% (w/v) insoluble polyvynilpyrrolidene (3:1; buffer volume : tissue on fresh weight). The homogenate was centrifuged at 10.000 rpm for 30 min at 4 °C and the supernatant was kept at -4 °C until needed for analysis.

3.2.4.1.1 Determination of peroxidase activity

The peroxidase activity was assayed by using Spectrophotometer (JENWAY 6305 UV/V) as described by **Amako *et al.* (1994)** using pyrogallol as a substrate and the absorbance was measured at 430 nm.

3.2.4.1.2 Determination of lipoxygenase activity

The lipoxygenase activity was assayed by using Spectrophotometer (JENWAY 6305 UV/V) as described by **Surry (1964)** using linoleic acid as a substrate and the absorbance was measured at 234 nm.

3.2.4.1.3 Determination of polyphenol oxidase activity

The polyphenol oxidase activity was assayed by using Spectrophotometer (JENWAY 6305 UV/V) as described by **Coseteng and Lee (1987)** using catechol as a substrate and the absorbance was measured at 420 nm.

3.2.5 Statistical methods

The data obtained in this study were subjected to statistical analysis by using Statistical Package for Social Science (SPSS). Means values were obtained by the Analysis of Variance (ANOVA) as described by **Mead and Gurnow (1985)**. Probability of 5% was used to indicate the significances by using Duncan's Multiple Range Test (DMRT) according to **Duncan's (1955)**.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Nutritional values of fresh vegetables

Table (4.1) shows the nutritional values of fresh okra, green beans, peas, spinach and molokhia samples. The moisture content in the different samples ranged from 83.86% in spinach to 86.52% in green beans, while, the available carbohydrates ranged from 4.13% in green beans to 6.83% in spinach. The highest value of crude fiber was found in peas (6.42%) while the lowest value was found in molokhia (5.64%). All the vegetable samples were found to be of very low levels of protein, ash and fat which ranged from 1.42 - 1.93%, 0.77 - 0.87% and 0.43 - 0.57%, respectively. The results obtained in this study are in accordance with **USDA (2012)**.

Moreover, all the vegetable samples were very rich in potassium, magnesium, calcium and sodium which ranged from 596.81 - 823.83, 564.45 - 783.26, 208.65 - 603.57 and 127.03 - 269.42 mg/100g, respectively. In addition to that, all the vegetable samples were found to be very rich in ascorbic acid which ranged from 38.13 in okra to 56.32 mg/100g in green beans. In contrast, the vegetables samples were very low in their energy contents which ranged from 27.66 in green beans to 39.70 kcal/100g in spinach. The results obtained in this study agrees well with those reported by **Mullen *et al.* (2002)** and **Chaovanalikit** and **Wrolstad (2004)** as they mentioned that vegetables are very useful in human diet because they are low in calories, protein and fat, but are very rich in vitamins, minerals, and fibers contents.

4.2 Hydrogen ions concentration (pH) and enzymatic activity in fresh vegetables

Table (4.2) presents the hydrogen ions concentration (pH) and enzymatic activity in fresh okra, green beans, peas, spinach and molokhia samples. The pH values in

Table (4.1): Nutritional values of fresh vegetables

Characteristics	Fresh vegetable samples				
	Okra	Green Beans	Peas	Spinach	Molokhia
	(n = 3 ± SD)				
Moisture %	084.87 ± 0.01^{a}	086.52 ± 0.03^{c}	084.43 ± 0.03^{d}	083.86 ± 0.01^{e}	084.78 ± 0.04^{b}
Protein %	001.42 ± 0.03^{c}	001.82 ± 0.05^{d}	001.93 ± 0.06^{a}	001.84 ± 0.03^{b}	001.46 ± 0.03^{e}
Fat %	000.46 ± 0.01^{d}	000.43 ± 0.03^{a}	000.47 ± 0.01^{c}	000.53 ± 0.01^{e}	000.57 ± 0.02^{b}
Total carbohydrates %	012.46 ± 0.05^{a}	010.37 ± 0.04^{b}	012.40 ± 0.06^{d}	012.94 ± 0.04^{c}	012.32 ± 0.01^{e}
Available carbohydrates %	006.54 ± 0.02^{e}	004.13 ± 0.05^{d}	005.98 ± 0.01^{a}	006.83 ± 0.03^{b}	006.68 ± 0.06^{c}
Crude fiber %	005.92 ± 0.06^{a}	006.24 ± 0.03^{c}	006.42 ± 0.03^{b}	006.11 ± 0.02^{d}	005.64 ± 0.04^{e}
Ash %	000.79 ± 0.03^{b}	000.86 ± 0.02^{d}	000.77 ± 0.04^{a}	000.83 ± 0.04^{e}	000.87 ± 0.03^{c}
Sodium (mg/100g)	226.27 ± 0.01^{a}	127.03 ± 0.01^{e}	218.63 ± 0.02^{c}	269.42 ± 0.06^{b}	163.48 ± 0.03^{d}
Potassium (mg/100g)	794.31 ± 0.03^{c}	712.43 ± 0.03^{d}	596.81 ± 0.04^{b}	795.46 ± 0.02^{e}	823.83 ± 0.04^{a}
Calcium (mg/100g)	402.56 ± 0.04^{e}	407.65 ± 0.06^{c}	407.57 ± 0.06^{a}	603.57 ± 0.06^{d}	208.65 ± 0.05^{b}
Magnesium (mg/100g)	678.72 ± 0.01^{c}	637.18 ± 0.01^{a}	564.45 ± 0.03^{d}	672.24 ± 0.02^{b}	783.26 ± 0.01^{e}
Ascorbic acid (mg/100g)	038.13 ± 0.06^{d}	056.32 ± 0.05^{c}	045.68 ± 0.03^{e}	052.47 ± 0.01^{a}	048.22 ± 0.05^{b}
Energy value: kcal/100g	036.29 ± 0.04^{c}	027.66 ± 0.06^{a}	036.04 ± 0.02^{d}	039.70 ± 0.05^{c}	037.94 ± 0.01^{e}
kj/100g	151.84 ± 0.02^{d}	115.72 ± 0.01^{e}	150.79 ± 0.04^{c}	166.09 ± 0.06^{a}	158.75 ± 0.05^{b}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.2): Hydrogen ions concentration (pH) and enzymatic activity in fresh vegetables

Characteristics	Fresh vegetable samples				
	Okra	Green Beans	Peas	Spinach	Molokhia
	(n = 3 ± SD)				
pH	5.93 ± 0.02^{c}	6.92 ± 0.03^{a}	6.93 ± 0.04^{d}	7.28 ± 0.04^{e}	7.57 ± 0.03^{b}
Peroxidase activity %	100	100	100	100	100
Lipoxygenase activity %	100	100	100	100	100
Polyphenol oxidase activity %	100	100	100	100	100

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

the different vegetable samples ranged from 5.93 in okra to 7.57 in molokhia. While, the enzymatic activities in the different fresh vegetable samples were found to be at the maximum levels (100%). The results obtained in this study are in a good agreement with those mentioned by **Sipos *et al.* (2009)**.

4.3 Changes in nutritional value, pH and enzymatic characteristics after processing

4.3.1 Changes in nutritional value of vegetables after blanching

Table (4.3) indicates the nutritional values of fresh okra, green beans, peas, spinach and molokhia samples after blanching step. The moisture content in the different vegetable samples slightly increased after blanching and ranged from 85.41% in spinach to 87.76% in green beans. The increment as percent was found to range from 1.43% in green beans to 1.84% in spinach. In contrast, the total carbohydrates slightly decreased and ranged from 9.32% in green beans to 11.78% in okra. The reduction as percent was estimated to range between 5.64% in okra to 10.13% in green beans. The highest value of crude fiber after washing and blanching was found in peas (5.81%), while the lowest value was found in molokhia (4.44%). All the vegetable samples after blanching step were found with very low levels of protein, ash and fat which ranged between 1.21 - 1.89%, 0.68 - 0.78% and 0.38 - 0.49%, respectively. The results obtained in this study are in a good agreement with those mentioned by **Savas *et al.* (2005)**.

On the other hand, all the vegetable samples were found to be with high levels of potassium, magnesium, calcium and sodium which ranged between 472.73 - 745.31, 482.52 - 613.11, 185.52 - 466.76 and 093.25 - 235.16 mg/100g, respectively. The vegetable samples were found to be very rich in ascorbic acid which ranged from 31.02 in okra to 46.38 mg/100g in spinach, but, very low in their energy contents which ranged from 24.90 in green beans to 37.13 kcal/100g

Table (4.3): Nutritional values of vegetables after blanching process

Characteristics	Blanched vegetable samples				
	Okra	Green Beans	Peas	Spinach	Molokhia
	(n = 3 ± SD)				
Moisture %	086.78 ± 0.03^{c}	087.76 ± 0.04^{e}	086.11 ± 0.02^{d}	085.41 ± 0.06^{b}	086.22 ± 0.03^{a}
Protein %	001.37 ± 0.01^{b}	001.76 ± 0.03^{d}	001.89 ± 0.04^{c}	001.68 ± 0.05^{a}	001.21 ± 0.04^{e}
Fat %	000.39 ± 0.04^{e}	000.38 ± 0.01^{c}	000.42 ± 0.02^{a}	000.47 ± 0.03^{d}	000.49 ± 0.01^{b}
Total carbohydrates %	011.78 ± 0.03^{c}	009.32 ± 0.02^{e}	010.90 ± 0.03^{d}	011.67 ± 0.02^{b}	011.32 ± 0.02^{a}
Available carbohydrates %	006.55 ± 0.04^{b}	003.62 ± 0.01^{d}	005.09 ± 0.02^{a}	006.26 ± 0.05^{c}	006.88 ± 0.03^{e}
Crude fiber %	005.23 ± 0.05^{a}	005.70 ± 0.01^{b}	005.81 ± 0.06^{e}	005.41 ± 0.04^{d}	004.44 ± 0.03^{c}
Ash %	000.69 ± 0.04^{e}	000.78 ± 0.04^{c}	000.68 ± 0.02^{a}	000.77 ± 0.03^{b}	000.76 ± 0.04^{d}
Sodium (mg/100g)	184.61 ± 0.02^{b}	093.25 ± 0.03^{d}	193.67 ± 0.03^{a}	235.16 ± 0.04^{e}	127.73 ± 0.05^{c}
Potassium (mg/100g)	676.64 ± 0.04^{d}	606.34 ± 0.05^{e}	472.73 ± 0.05^{c}	643.83 ± 0.01^{d}	745.31 ± 0.01^{a}
Calcium (mg/100g)	326.48 ± 0.01^{a}	302.52 ± 0.03^{b}	312.17 ± 0.03^{d}	466.76 ± 0.03^{c}	185.52 ± 0.01^{e}
Magnesium (mg/100g)	561.29 ± 0.05^{e}	558.71 ± 0.04^{c}	482.52 ± 0.02^{a}	585.62 ± 0.01^{b}	613.11 ± 0.02^{d}
Ascorbic acid (mg/100g)	031.02 ± 0.03^{c}	044.85 ± 0.01^{e}	039.71 ± 0.04^{d}	046.38 ± 0.02^{a}	039.03 ± 0.03^{b}
Energy value: kcal/100g	035.55 ± 0.06^{b}	024.90 ± 0.03^{a}	031.80 ± 0.05^{e}	036.23 ± 0.01^{d}	037.13 ± 0.02^{c}
kj/100g	148.75 ± 0.01^{d}	104.21 ± 0.05^{b}	133.05 ± 0.02^{c}	151.57 ± 0.06^{e}	155.35 ± 0.03^{a}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

in molokhia. The results obtained in this study agree well with those reported by **Van-den-Berg *et al.* (2000)**.

4.3.2 Changes in hydrogen ions concentration (pH) and enzymatic activity after blanching

Table (4.4) represents the hydrogen ions concentration (pH) and enzymatic activity in blanched okra, green beans, peas, spinach and molokhia samples. The pH values in the different vegetable samples ranged from 5.86 in okra to 7.46 in molokhia. Whereas, the activities of lipoxygenase, polyphenol oxidase and peroxidase enzymes ranged from 8.54 - 9.97%, 9.03 - 9.92% and 8.68 - 9.87%, respectively after blanching. The results obtained in this study are in agreement with those stated by **Murcia *et al.* (2000); Martinis** and **Silva (2004)** and **Savas *et al.* (2005)**. The blanching process was mentioned by the previous authors to inactivate the different enzymes that cause undesirable changes in vegetables color, flavor, and nutritional value during frozen storage period.

4.3.3 Changes in nutritional value of vegetables after freezing

Table (4.5) represents the nutritional values of fresh okra, green beans, peas, spinach and molokhia samples after freezing. The moisture content in the different vegetable samples were slightly decreased after the freezing process and ranged from 84.69% in spinach to 87.68% in green beans. The reduction as percent was estimated to range from 0.09% in green beans to 0.84% in spinach. In contrast, the available carbohydrates were slightly increased and ranged from 4.06% in green beans to 7.61% in molokhia. The increment as percent range from 10.61% in molokhia to 12.15% in green beans. The highest value of crude fiber was found in green beans (5.43%), while, the lowest value was found in molokhia (4.12%).

However, all the vegetable samples were found with very low levels of protein, ash and fat which ranged between 1.18 - 1.83%, 0.64 - 0.75% and 0.35 - 0.46%, respectively. In contrast, all the vegetable samples were very rich in

Table (4.4): Hydrogen ions concentration (pH) and enzymatic activity in blanched vegetables

Characteristics	Blanched vegetable samples				
	Okra	Green Beans	Peas	Spinach	Molokhia
	(n = 3 ± SD)				
pH	005.86 ± 0.03^{d}	006.76 ± 0.02^{b}	006.85 ± 0.03^{e}	007.12 ± 0.05^{a}	007.46 ± 0.04^{c}
Peroxidase activity %	009.45 ± 0.01^{b}	009.11 ± 0.03^{e}	009.87 ± 0.04^{d}	008.68 ± 0.04^{c}	009.73 ± 0.02^{a}
Lipoxygenase activity %	009.97 ± 0.04^{c}	009.66 ± 0.02^{a}	009.42 ± 0.02^{b}	008.94 ± 0.02^{d}	008.54 ± 0.04^{e}
Polyphenol oxidase activity %	009.92 ± 0.03^{e}	009.85 ± 0.04^{c}	009.26 ± 0.03^{a}	009.03 ± 0.01^{b}	009.48 ± 0.03^{d}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.5): Nutritional values of vegetables after freezing process

Characteristics	Frozen vegetable samples				
	Okra	Green Beans	Peas	Spinach	Molokhia
	(n = 3 ± SD)				
Moisture %	086.32 ± 0.04^{e}	087.68 ± 0.05^{b}	085.36 ± 0.04^{a}	084.69 ± 0.04^{d}	085.90 ± 0.02^{c}
Protein %	001.32 ± 0.06^{d}	001.72 ± 0.06^{a}	001.83 ± 0.01^{c}	001.62 ± 0.01^{e}	001.18 ± 0.03^{b}
Fat %	000.35 ± 0.02^{b}	000.36 ± 0.04^{e}	000.39 ± 0.04^{a}	000.43 ± 0.02^{c}	000.46 ± 0.04^{d}
Total carbohydrates %	011.34 ± 0.01^{c}	009.49 ± 0.05^{a}	011.78 ± 0.02^{d}	012.52 ± 0.01^{b}	011.73 ± 0.03^{e}
Available carbohydrates %	006.40 ± 0.03^{d}	004.06 ± 0.01^{e}	006.46 ± 0.04^{c}	007.39 ± 0.02^{a}	007.61 ± 0.05^{b}
Crude fiber %	004.94 ± 0.03^{e}	005.43 ± 0.02^{c}	005.32 ± 0.01^{b}	005.03 ± 0.03^{d}	004.12 ± 0.06^{a}
Ash %	000.67 ± 0.01^{b}	000.75 ± 0.01^{d}	000.64 ± 1.06^{a}	000.74 ± 0.01^{c}	000.73 ± 0.01^{e}
Sodium (mg/100g)	166.83 ± 0.05^{e}	085.38 ± 0.04^{c}	174.64 ± 0.04^{b}	211.84 ± 0.03^{d}	106.32 ± 0.04^{a}
Potassium (mg/100g)	656.37 ± 0.01^{c}	586.53 ± 0.01^{a}	418.39 ± 0.03^{d}	605.31 ± 0.04^{b}	711.64 ± 0.03^{e}
Calcium (mg/100g)	309.59 ± 0.06^{e}	292.64 ± 0.02^{d}	301.83 ± 0.05^{c}	403.59 ± 0.02^{a}	171.85 ± 0.03^{b}
Magnesium (mg/100g)	556.42 ± 0.03^{d}	551.27 ± 0.03^{c}	474.72 ± 0.01^{a}	578.72 ± 0.04^{b}	604.24 ± 0.04^{e}
Ascorbic acid (mg/100g)	026.57 ± 0.02^{e}	038.68 ± 0.03^{b}	037.53 ± 0.05^{c}	044.16 ± 0.04^{a}	034.60 ± 0.02^{d}
Energy value: kcal/100g	034.41 ± 0.04^{c}	026.40 ± 0.06^{a}	036.96 ± 0.01^{d}	040.31 ± 0.05^{b}	039.77 ± 0.03^{e}
kj/100g	143.95 ± 0.06^{a}	110.44 ± 0.01^{d}	154.65 ± 0.03^{e}	168.70 ± 0.04^{c}	166.40 ± 0.05^{b}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

potassium, magnesium, calcium, sodium and ascorbic acid which ranged between 418.39 - 711.64, 474.72 - 604.24, 171.85 - 403.59, 085.38 - 211.84 and 26.57 - 44.16 mg/100g, respectively. The results obtained in this study are in accordance to those reported by **Giannakourou** and **Taoukis (2003)**. In general, the vegetables samples were found very low in their energy contents which ranged from 26.40 in green beans to 40.31 kcal/100g in spinach. The results obtained in this study are in agreement with those stated by **Mullen *et al.* (2002); Chaovanalikit** and **Wrolstad (2004); Czarnowska** and **Gujska (2012)**.

4.3.4 Changes in hydrogen ions concentration (pH) and enzymatic activity after freezing

Table (4.6) indicates the hydrogen ions concentration (pH) and enzymatic activity in frozen okra, green beans, peas, spinach and molokhia samples. The pH values in the different vegetable samples ranged from 5.79 in okra to 7.33 in molokhia, while the activities of lipoxygenase, peroxidase and polyphenol oxidase ranged between 8.38 - 9.86%, 8.58 - 9.84% and 8.87 - 9.81%, respectively in the different vegetables after freezing. From the results obtained in this study, both the hydrogen ions concentration (pH) and enzymatic activity in the different vegetable samples were found to decrease slightly during the freezing process in comparison with the blanched vegetable samples. The results obtained in this study are in agreement with those reported by **Savas *et al.* (2005)** and **Sipos *et al.* (2009)**.

4.4 Effects of blanching and freezing processes on vegetables nutritional values

4.4.1 Okra

Table (4.7) and (4.8) present the effects of blanching and freezing processes on okra nutritional value. The results show that, after blanching process the moisture content and available carbohydrates were significantly increased by

Table (4.6): Hydrogen ions concentration (pH) and enzymatic activity in frozen vegetables

Characteristics	Frozen vegetable samples				
	Okra	Green Beans	Peas	Spinach	Molokhia
	(n = 3 ± SD)				
pH	005.79 ± 0.01^{e}	006.64 ± 0.06^{b}	006.79 ± 0.02^{d}	006.98 ± 0.02^{c}	007.33 ± 0.01^{a}
Peroxidase activity %	009.32 ± 0.05^{a}	009.00 ± 0.06^{d}	009.84 ± 0.05^{e}	008.58 ± 0.03^{d}	009.57 ± 0.04^{c}
Lipoxygenase activity %	009.86 ± 0.03^{b}	009.55 ± 0.04^{a}	009.29 ± 0.03^{d}	008.76 ± 0.05^{c}	008.38 ± 0.05^{e}
Polyphenol oxidase activity %	009.81 ± 0.02^{e}	009.62 ± 0.01^{c}	009.16 ± 0.02^{b}	008.87 ± 0.02^{a}	009.38 ± 0.01^{d}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.7): Effects of blanching and freezing processes on okra nutritional value

Characteristics	Okra		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
Moisture %	084.87 ± 0.01[a]	086.78 ± 0.03[c]	086.32 ± 0.04[b]
Protein %	001.42 ± 0.03[c]	001.37 ± 0.01[b]	001.32 ± 0.06[a]
Fat %	000.46 ± 0.01[c]	000.39 ± 0.04[a]	000.35 ± 0.02[b]
Total carbohydrates %	012.46 ± 0.05[a]	011.78 ± 0.03[b]	011.34 ± 0.01[c]
Available carbohydrates %	006.54 ± 0.02[c]	006.55 ± 0.04[b]	006.40 ± 0.03[a]
Crude fiber %	005.92 ± 0.06[b]	005.23 ± 0.05[a]	004.94 ± 0.03[c]
Ash %	000.79 ± 0.03[a]	000.69 ± 0.04[c]	000.67 ± 0.01[b]
Sodium (mg/100g)	226.27 ± 0.01[c]	184.61 ± 0.02[b]	166.83 ± 0.05[a]
Potassium (mg/100g)	794.31 ± 0.03[b]	676.64 ± 0.04[a]	656.37 ± 0.01[c]
Calcium (mg/100g)	402.56 ± 0.04[a]	326.48 ± 0.01[c]	309.59 ± 0.06[b]
Magnesium (mg/100g)	678.72 ± 0.01[c]	561.29 ± 0.05[b]	556.42 ± 0.03[a]
Ascorbic acid (mg/100g)	038.13 ± 0.06[a]	031.02 ± 0.03[c]	026.57 ± 0.02[b]
Energy value: kcal/100g	036.29 ± 0.04[c]	035.55 ± 0.06[b]	034.41 ± 0.04[a]
kj/100g	151.84 ± 0.02[b]	148.75 ± 0.01[a]	143.95 ± 0.06[c]

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.8): Changes in okra nutritional value after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
Energy value (kcal/100g)	036.29	– 02.04	– 03.31	– 05.35
Moisture %	084.87	+ 02.25	– 00.53	+ 01.72
Available carbohydrates %	006.54	+ 00.15	– 02.29	– 02.14
Ascorbic acid (mg/100g)	038.13	– 18.65	– 14.35	– 33.00
Sodium (mg/100g)	226.27	– 18.41	– 09.63	– 28.04
Potassium (mg/100g)	794.31	– 14.81	– 03.00	– 17.81
Calcium (mg/100g)	402.56	– 18.90	– 05.17	– 13.73
Magnesium (mg/100g)	678.72	– 17.30	– 00.87	– 18.17

2.25% and 0.15%, respectively, while, sodium, potassium, calcium, magnesium, ascorbic acid and energy content were decreased by 18.41%, 14.81%, 18.90%, 17.30%, 18.65% and 2.04%, respectively.

But, after freezing process the moisture content and available carbohydrates were decreased by 0.53% and 2.29%, respectively. While, sodium, potassium, calcium, magnesium, ascorbic acid and energy value were continued to decrease by 9.63%, 3.00%, 5.17%, 0.87%, 14.35% and 3.31%, respectively in comparison with their values after the blanching step. The results obtained in this study agree well with those reported by **Van-den-Berg *et al.* (2000)** and **Savas *et al.* (2005)**.

4.4.2 Green beans

Table (4.9) and (4.10) show the effects of blanching and freezing process on green beans nutritional value. The results show that, after blanching process the moisture content slightly increased by 1.34%, while, the available carbohydrates, sodium, potassium, calcium, magnesium, ascorbic acid and energy content significantly (≤ 0.05) decreased by 12.35%, 26.59%, 14.89%, 25.79%, 12.31%, 20.37% and 9.98%, respectively. This may be attributed to the increment of green beans moisture content.

But, after the freezing processes the available carbohydrates and energy content in green beans significantly (≤ 0.05) increased by 12.15% and 6.02%. In contrast, the moisture, sodium, potassium, calcium, magnesium, and ascorbic acid contents were decreased by 0.09%, 8.44%, 3.27%, 3.27%, 1.33% and 13.76%, respectively, in comparison with the values of the blanched samples. The results obtained in this study agrees well with those stated by **Giannakourou** and **Taoukis (2003); Savas *et al.* (2005); Czarnowska** and **Gujska (2012)**.

Table (4.9): Effects of blanching and freezing processes on green beans nutritional value

Characteristics	Green beans		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
Moisture %	086.52 ± 0.03^{c}	087.76 ± 0.04^{a}	087.68 ± 0.05^{b}
Protein %	001.82 ± 0.05^{b}	001.76 ± 0.03^{c}	001.72 ± 0.06^{a}
Fat %	000.43 ± 0.03^{a}	000.38 ± 0.01^{c}	000.36 ± 0.04^{b}
Total carbohydrates %	010.37 ± 0.04^{b}	009.32 ± 0.02^{a}	009.49 ± 0.05^{c}
Available carbohydrates %	004.13 ± 0.05^{a}	003.62 ± 0.01^{c}	004.06 ± 0.01^{b}
Crude fiber %	006.24 ± 0.03^{c}	005.70 ± 0.01^{b}	005.43 ± 0.02^{a}
Ash %	000.86 ± 0.02^{a}	000.78 ± 0.04^{c}	000.75 ± 0.01^{b}
Sodium (mg/100g)	127.03 ± 0.01^{b}	093.25 ± 0.03^{a}	085.38 ± 0.04^{c}
Potassium (mg/100g)	712.43 ± 0.03^{a}	606.34 ± 0.05^{c}	586.53 ± 0.01^{b}
Calcium (mg/100g)	407.65 ± 0.06^{c}	302.52 ± 0.03^{b}	292.64 ± 0.02^{a}
Magnesium (mg/100g)	637.18 ± 0.01^{a}	558.71 ± 0.04^{c}	551.27 ± 0.03^{b}
Ascorbic acid (mg/100g)	056.32 ± 0.05^{c}	044.85 ± 0.01^{b}	038.68 ± 0.03^{a}
Energy value: kcal/100g	027.66 ± 0.06^{b}	024.90 ± 0.03^{a}	026.40 ± 0.06^{c}
kj/100g	115.72 ± 0.01^{c}	104.21 ± 0.05^{b}	110.44 ± 0.01^{a}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.10): Changes in green beans nutritional value after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
Energy value (kcal/100g)	036.29	– 09.98	+ 06.02	– 03.96
Moisture %	084.87	+ 01.43	– 00.09	+ 01.34
Available carbohydrates %	006.54	– 12.35	+ 12.15	– 00.20
Ascorbic acid (mg/100g)	038.13	– 20.37	– 13.76	– 34.13
Sodium (mg/100g)	226.27	– 26.59	– 08.44	– 35.03
Potassium (mg/100g)	794.31	– 14.89	– 03.27	– 18.16
Calcium (mg/100g)	402.56	– 25.79	– 03.27	– 29.06
Magnesium (mg/100g)	678.72	– 12.31	– 01.33	– 13.64

4.4.3 Peas

Table (4.11) and (4.12) represent the effects of blanching and freezing processes on peas nutritional value. The results indicate that, after the blanching process the moisture content was slightly increased by 1.98%, while, the available carbohydrates, sodium, potassium, calcium, magnesium, ascorbic acid and energy contents were significantly (≤ 0.05) decreased by 14.88%, 11.42%, 20.79%, 23.41%, 14.51%, 13.07% and 11.76%, respectively. The decrease in the different nutrients in peas can be also attributed to the increment in peas moisture content after washing and blanching.

But, after the freezing process the available carbohydrates and energy contents significantly (≤ 0.05) increased by 26.91% and 16.23%, respectively, the protein, fat, crude fiber, ash, sodium, potassium, calcium, magnesium, and ascorbic acid significantly (≤ 0.05) decreased by 9.83%, 11.49%, 3.31%, 1.62% and 5.49%, respectively. The results obtained in this study are in a good agreement with those reported by **Van-den-Berg *et al.* (2000); Giannakourou** and **Taoukis (2003); Czarnowska** and **Gujska (2012)**.

4.4.4 Spinach

Table (4.13) and (4.14) indicate the effects of blanching and freezing processes on spinach nutritional value. The results show that, after the blanching process the moisture content slightly increased by 1.84%. While, the available carbohydrates, sodium, potassium, calcium, magnesium, ascorbic acid and energy content were significantly (≤ 0.05) decreased by 8.34%, 12.72%, 19.06%, 22.67%, 12.88%, 11.61% and 8.74%, respectively. The decrease in the different nutrients can be attributed to the increment in spinach moisture content.

On the other hand, after freezing of spinach, the available carbohydrates and energy contents significantly (≤ 0.05) increased by 18.05% and 11.26%, respectively, due to the decrease in spinach moisture content. While, sodium,

Table (4.11): Effects of blanching and freezing processes on peas nutritional value

Characteristics	Peas		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
Moisture %	084.43 ± 0.03^{c}	086.11 ± 0.02^{b}	085.36 ± 0.04^{a}
Protein %	001.93 ± 0.06^{a}	001.89 ± 0.04^{c}	001.83 ± 0.01^{b}
Fat %	000.47 ± 0.01^{b}	000.42 ± 0.02^{a}	000.39 ± 0.04^{c}
Total carbohydrates %	012.40 ± 0.06^{c}	010.90 ± 0.03^{b}	011.78 ± 0.02^{a}
Available carbohydrates %	005.98 ± 0.01^{b}	005.09 ± 0.02^{a}	006.46 ± 0.04^{c}
Crude fiber %	006.42 ± 0.03^{a}	005.81 ± 0.06^{c}	005.32 ± 0.01^{b}
Ash %	000.77 ± 0.04^{b}	000.68 ± 0.02^{a}	000.64 ± 1.06^{c}
Sodium (mg/100g)	218.63 ± 0.02^{c}	193.67 ± 0.03^{b}	174.64 ± 0.04^{a}
Potassium (mg/100g)	596.81 ± 0.04^{a}	472.73 ± 0.05^{c}	418.39 ± 0.03^{b}
Calcium (mg/100g)	407.57 ± 0.06^{a}	312.17 ± 0.03^{b}	301.83 ± 0.05^{c}
Magnesium (mg/100g)	564.45 ± 0.03^{b}	482.52 ± 0.02^{c}	474.72 ± 0.01^{a}
Ascorbic acid (mg/100g)	045.68 ± 0.03^{a}	039.71 ± 0.04^{b}	037.53 ± 0.05^{c}
Energy value: kcal/100g	036.04 ± 0.02^{b}	031.80 ± 0.05^{c}	036.96 ± 0.01^{a}
kj/100g	150.79 ± 0.04^{c}	133.05 ± 0.02^{a}	154.65 ± 0.03^{b}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.12): Changes in peas nutritional value after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
Energy value (kcal/100g)	036.29	– 11.76	+ 16.23	+ 04.47
Moisture %	084.87	+ 01.98	– 00.87	+ 01.11
Available carbohydrates %	006.54	– 14.88	+ 26.91	+ 12.03
Ascorbic acid (mg/100g)	038.13	– 13.07	– 05.49	– 18.56
Sodium (mg/100g)	226.27	– 11.42	– 09.83	– 21.25
Potassium (mg/100g)	794.31	– 20.79	– 11.49	– 32.28
Calcium (mg/100g)	402.56	– 23.41	– 03.31	– 26.72
Magnesium (mg/100g)	678.72	– 14.51	– 01.62	– 16.13

Table (4.13): Effects of blanching and freezing processes on spinach nutritional value

Characteristics	Spinach		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
Moisture %	083.86 ± 0.01^{c}	085.41 ± 0.06^{b}	084.69 ± 0.04^{a}
Protein %	001.84 ± 0.03^{b}	001.68 ± 0.05^{a}	001.62 ± 0.01^{c}
Fat %	000.53 ± 0.01^{c}	000.47 ± 0.03^{b}	000.43 ± 0.02^{a}
Total carbohydrates %	012.94 ± 0.04^{c}	011.67 ± 0.02^{a}	012.52 ± 0.01^{b}
Available carbohydrates %	006.83 ± 0.03^{b}	006.26 ± 0.05^{c}	007.39 ± 0.02^{a}
Crude fiber %	006.11 ± 0.02^{a}	005.41 ± 0.04^{b}	005.03 ± 0.03^{c}
Ash %	000.83 ± 0.04^{b}	000.77 ± 0.03^{c}	000.74 ± 0.01^{a}
Sodium (mg/100g)	269.42 ± 0.06^{a}	235.16 ± 0.04^{b}	211.84 ± 0.03^{c}
Potassium (mg/100g)	795.46 ± 0.02^{c}	643.83 ± 0.01^{a}	605.31 ± 0.04^{b}
Calcium (mg/100g)	603.57 ± 0.06^{b}	466.76 ± 0.03^{c}	403.59 ± 0.02^{a}
Magnesium (mg/100g)	672.24 ± 0.02^{a}	585.62 ± 0.01^{b}	578.72 ± 0.04^{c}
Ascorbic acid (mg/100g)	052.47 ± 0.01^{b}	046.38 ± 0.02^{c}	044.16 ± 0.04^{a}
Energy value: kcal/100g	039.70 ± 0.05^{c}	036.23 ± 0.01^{a}	040.31 ± 0.05^{b}
kj/100g	166.09 ± 0.06^{a}	151.57 ± 0.06^{b}	168.70 ± 0.04^{c}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.14): Changes in spinach nutritional value after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
Energy value (kcal/100g)	036.29	– 08.74	+ 11.26	+ 02.52
Moisture %	084.87	+ 01.84	– 00.84	+ 01.00
Available carbohydrates %	006.54	– 08.34	+ 18.05	+ 09.71
Ascorbic acid (mg/100g)	038.13	– 11.61	– 04.79	– 16.40
Sodium (mg/100g)	226.27	– 12.72	– 09.92	– 22.64
Potassium (mg/100g)	794.31	– 19.06	– 05.98	– 25.04
Calcium (mg/100g)	402.56	– 22.67	– 13.53	– 36.20
Magnesium (mg/100g)	678.72	– 12.88	– 01.11	– 13.99

potassium, calcium, magnesium, and ascorbic acid were decreased by 9.92%, 5.98%, 13.53%, 1.11% and 4.79%, respectively. The results obtained in this study are in accordance with those reported by **Giannakourou** and **Taoukis (2003)** and **Savas *et al.* (2005)**.

4.4.5 Molokhia

Table (4.15) and (4.16) present the effects of blanching and freezing processes on molokhia nutritional value. The results show that, after the blanching process the moisture content and available carbohydrates were slightly increased by 1.70% and 2.99%, respectively, while, sodium, potassium, calcium, magnesium, ascorbic acid and energy contents were significantly ($\leq$ 0.05) decreased by 21.87%, 9.53%, 11.08%, 21.72%, 19.06% and 1.69%, respectively, due to the increment in molokhia moisture and available carbohydrates contents.

Also, after the freezing process the available carbohydrates and energy contents significantly ($\leq$ 0.05) increased by 10.61% and 7.11%, respectively, while, moisture, sodium, potassium, calcium, magnesium, and ascorbic acid were significantly ($\leq$ 0.05) decreased by 0.37%, 16.76%, 4.52%, 7.37%, 1.45% and 11.35%, respectively. The results obtained in this study agree well with those mentioned by **Van-den-Berg *et al.* (2000); Czarnowska** and **Gujska (2012)**. The latter author mentioned that, although the freezing process is regarded as the simplest and the most important preservation method for vegetables, some vitamins and minerals may be lost during the process.

4.5 Effects of blanching and freezing processes on vegetables hydrogen ions concentration (pH) and enzymatic activity

4.5.1 Okra

Table (4.17) indicates the effects of blanching and freezing processes on okra hydrogen ions concentration (pH) and the activities of peroxidase,

Table (4.15): Effects of blanching and freezing processes on molokhia nutritional value

Characteristics	Molokhia		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
Moisture %	084.78 ± 0.04^{b}	086.22 ± 0.03^{a}	085.90 ± 0.02^{c}
Protein %	001.46 ± 0.03^{c}	001.21 ± 0.04^{b}	001.18 ± 0.03^{a}
Fat %	000.57 ± 0.02^{a}	000.49 ± 0.01^{c}	000.46 ± 0.04^{b}
Total carbohydrates %	012.32 ± 0.01^{b}	011.32 ± 0.02^{a}	011.73 ± 0.03^{c}
Available carbohydrates %	006.68 ± 0.06^{c}	006.88 ± 0.03^{b}	007.61 ± 0.05^{a}
Crude fiber %	005.64 ± 0.04^{a}	004.44 ± 0.03^{c}	004.12 ± 0.06^{b}
Ash %	000.87 ± 0.03^{b}	000.76 ± 0.04^{a}	000.73 ± 0.01^{c}
Sodium (mg/100g)	163.48 ± 0.03^{c}	127.73 ± 0.05^{b}	106.32 ± 0.04^{a}
Potassium (mg/100g)	823.83 ± 0.04^{b}	745.31 ± 0.01^{a}	711.64 ± 0.03^{c}
Calcium (mg/100g)	208.65 ± 0.05^{a}	185.52 ± 0.01^{c}	171.85 ± 0.03^{b}
Magnesium (mg/100g)	783.26 ± 0.01^{c}	613.11 ± 0.02^{b}	604.24 ± 0.04^{a}
Ascorbic acid (mg/100g)	048.22 ± 0.05^{b}	039.03 ± 0.03^{a}	034.60 ± 0.02^{c}
Energy value: kcal/100g	037.94 ± 0.01^{a}	037.13 ± 0.02^{b}	039.77 ± 0.03^{c}
kj/100g	158.75 ± 0.05^{c}	155.35 ± 0.03^{a}	166.40 ± 0.05^{b}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.16): Changes in molokhia nutritional value after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
Energy value (kcal/100g)	036.29	– 01.69	+ 07.11	+ 05.42
Moisture %	084.87	+ 01.70	– 00.37	+ 01.33
Available carbohydrates %	006.54	+ 02.99	+ 10.61	+ 11.06
Ascorbic acid (mg/100g)	038.13	– 19.06	– 11.35	– 22.41
Sodium (mg/100g)	226.27	– 21.87	– 16.76	– 38.63
Potassium (mg/100g)	794.31	– 09.53	– 04.52	– 14.05
Calcium (mg/100g)	402.56	– 11.08	– 07.37	– 18.45
Magnesium (mg/100g)	678.72	– 21.72	– 01.45	– 23.17

Table (4.17): Effects of blanching and freezing processes on okra hydrogen ions concentration (pH) and enzymatic activity

Characteristics	Okra		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
pH	5.93 ± 0.02[a]	5.86 ± 0.03[b]	5.79 ± 0.01[c]
Peroxidase activity %	100	9.45 ± 0.01[a]	9.32 ± 0.05[b]
Lipoxygenase activity %	100	9.97 ± 0.04[b]	9.86 ± 0.03[a]
Polyphenol oxidase activity %	100	9.92 ± 0.03[b]	9.81 ± 0.02[a]

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

lipoxygenase and polyphenol oxidase enzymes. After the blanching process the pH and the activities of the aforementioned enzymes were found to decrease by 1.18%, 90.55%, 90.03% and 90.08%, respectively as shown in Table (4.18). But after the freezing process, the pH and the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes were slightly decreased by 1.19%, 1.38%, 1.10% and 1.10%, respectively. The results obtained in this study agree well with those reported by **Martinis** and **Silva (2004)** and **Savas *et al.* (2005)**.

4.5.2 Green beans

Table (4.19) represents the effects of blanching and freezing processes on green beans hydrogen ions concentration (pH) and enzymatic activity. The results show that, the peroxidase, lipoxygenase and polyphenol oxidase activities were greatly decreased after the blanching process by 90.89%, 90.34% and 90.15%, respectively as indicated in Table (4.20). But, after the freezing process, the pH and the activities of the previously mentioned enzymes slightly decreased by 1.77%, 1.21%, 1.14% and 2.33%, respectively in comparison with those values of the blanched samples. The results obtained in this study are in a good agreement with those reported by **Martinis** and **Silva (2004)** and **Sipos *et al.* (2009)**.

4.5.3 Peas

Table (4.21) shows the effects of blanching and freezing processes on peas hydrogen ions concentration (pH) and enzymatic activity. The results show that, the activites of peroxidase, lipoxygenase and polyphenol oxidase enzymes, significantly (≤ 0.05) decreased by 90.03%, 90.58% and 90.74%, respectively after the blanching process (Table, 4.22). But, after the freezing process the pH and the activities of the aforesaid enzymes were slightly decreased by 0.88%, 1.30%, 1.38% and 1.08%. The results obtained in this study agree well with those stated by **Savas *et al.* (2005)** and **Sipos *et al.* (2009)**.

Table (4.18): Changes in okra pH and enzyme activity after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
pH	005.93	– 01.18	– 01.19	– 02.37
Peroxidase activity %	100	– 90.55	– 01.38	– 91.93
Lipoxygenase activity %	100	– 90.03	– 01.10	– 91.13
Polyphenol oxidase activity %	100	– 90.08	– 01.10	– 91.18

Table (4.19): Effects of blanching and freezing processes on green beans hydrogen ions concentration (pH) and enzymatic activity

Characteristics	Green beans		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
pH	6.92 ± 0.03[a]	6.76 ± 0.02[b]	6.64 ± 0.06[c]
Peroxidase activity %	100	9.11 ± 0.03[a]	9.00 ± 0.06[b]
Lipoxygenase activity %	100	9.66 ± 0.02[b]	9.55 ± 0.04[a]
Polyphenol oxidase activity %	100	9.85 ± 0.04[a]	9.62 ± 0.01[b]

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.20): Changes in green beans pH and enzyme activity after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
pH	005.93	– 02.31	– 01.77	– 04.08
Peroxidase activity %	100	– 90.89	– 01.21	– 92.10
Lipoxygenase activity %	100	– 90.34	– 01.14	– 91.48
Polyphenol oxidase activity %	100	– 90.15	– 02.33	– 92.48

Table (4.21): Effects of blanching and freezing processes on peas hydrogen ions concentration (pH) and enzymatic activity

Characteristics	Peas		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
pH	6.93 ± 0.04[a]	6.85 ± 0.03[c]	6.79 ± 0.02[b]
Peroxidase activity %	100	9.97 ± 0.04[a]	9.84 ± 0.05[b]
Lipoxygenase activity %	100	9.42 ± 0.02[b]	9.29 ± 0.03[a]
Polyphenol oxidase activity %	100	9.26 ± 0.03[a]	9.16 ± 0.02[b]

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.22): Changes in peas pH and enzyme activity after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
pH	005.93	– 01.15	– 00.88	– 02.03
Peroxidase activity %	100	– 90.03	– 01.30	– 91.33
Lipoxygenase activity %	100	– 90.58	– 01.38	– 91.96
Polyphenol oxidase activity %	100	– 90.74	– 01.08	– 91.82

4.5.4 Spinach

Table (4.23) shows the effects of blanching and freezing processes on spinach hydrogen ions concentration (pH) and enzymatic activity. The results indicate that, the blanching process was greatly affected by the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes which were decreased by 91.32%, 91.06% and 90.97%, respectively in comparison with their values in the frozen samples (Table, 4.24). But, slight changes were found after the freezing process with respect to spinach pH and the activities of the aforementioned enzymes which were found to decrease by only 1.97%, 1.15%, 2.01% and 1.77%, respectively. The results obtained in this study are in accordance with results obtained by **Martinis** and **Silva (2004)** and **Sipos *et al.* (2009)**.

4.5.5 Molokhia

Table (4.25) represents the effects of blanching and freezing processes on molokhia hydrogen ions concentration (pH) and enzymatic activity. The results indicate that, after the blanching process the pH slightly decreased from 6.93 to 6.85, while, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes were significantly (≤ 0.05) decreased by 90.27%, 91.46% and 90.52%, respectively (Table, 4.26). But, the pH value and the activities of the previously mentioned enzymes were slightly decreased by 1.74%, 1.64%, 1.87% and 1.05%, respectively after the freezing process, in comparison with their values in the blanched samples. The results obtained in this study agree well with those reported by **Savas *et al.* (2005)** and **Sipos *et al.* (2009)**.

Table (4.23): Effects of blanching and freezing processes on spinach hydrogen ions concentration (pH) and enzymatic activity

Characteristics	Spinach		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
pH	7.28 ± 0.04^{c}	7.12 ± 0.05^{a}	6.98 ± 0.02^{b}
Peroxidase activity %	100	8.68 ± 0.04^{b}	8.58 ± 0.03^{a}
Lipoxygenase activity %	100	8.94 ± 0.02^{a}	8.76 ± 0.05^{b}
Polyphenol oxidase activity %	100	9.03 ± 0.01^{a}	8.87 ± 0.02^{b}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.24): Changes in spinach pH and enzyme activity after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
pH	005.93	– 02.20	– 01.97	– 04.17
Peroxidase activity %	100	– 91.32	– 01.15	– 92.47
Lipoxygenase activity %	100	– 91.06	– 02.01	– 93.07
Polyphenol oxidase activity %	100	– 90.97	– 01.77	– 92.74

Table (4.25): Effects of blanching and freezing processes on molokhia hydrogen ions concentration (pH) and enzymatic activity

Characteristics	Molokhia		
	Fresh sample	After blanching step	After freezing step
	(n = 3 ± SD)		
pH	7.57 ± 0.03^{b}	7.46 ± 0.04^{c}	7.33 ± 0.01^{a}
Peroxidase activity %	100	9.73 ± 0.02^{a}	9.57 ± 0.04^{b}
Lipoxygenase activity %	100	8.54 ± 0.04^{a}	8.38 ± 0.05^{b}
Polyphenol oxidase activity %	100	9.48 ± 0.03^{b}	9.38 ± 0.01^{a}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.26): Changes in molokhia pH and enzyme activity after blanching and freezing steps as percentage

Characteristics	Initial value	Changes as %		
		After blanching step	After freezing step	Total effect
pH	005.93	– 01.45	– 01.74	– 03.19
Peroxidase activity %	100	– 90.27	– 01.64	– 91.91
Lipoxygenase activity %	100	– 91.46	– 01.87	– 93.33
Polyphenol oxidase activity %	100	– 90.52	– 01.05	– 91.57

4.6 Effects of frozen storage on vegetables nutritional value and their quality characteristics

4.6.1 Okra

Table (4.27) indicates the effects of frozen storage on okra nutritional value, pH and enzymatic activity. The results show that, after a frozen storage period of 12 months at -18°C, the okra energy content and its available carbohydrates were significantly increased by 24.18% and 35.94%, respectively, while, its moisture, ascorbic acid, sodium, potassium, calcium, magnesium, pH, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes were significantly decreased by 2.08%, 4.59%, 23.02%, 8.19%, 18.36%, 13.82%, 21.07%, 37.77%, 31.95% and 28.85%, respectively as indicated in Table (4.28). The increment in okra energy value as well as the decrease in okra ascorbic acid and minerals content may be due to the increment in okra available carbohydrates as a result of biochemical degradation during the storage period. Figure (4.1) indicates the effects of frozen storage on okra minerals content. However, the results obtained in this study are in a good agreement with those mentioned by **Puupponen- Pimiä *et al.* (2003); Sahari *et al.* (2004)** and **Matkowski (2009)**.

4.6.2 Green beans

Table (4.29) represents the effects of frozen storage on green beans nutritional value, pH and enzymatic activity. The results show that, after a frozen storage period of 12 months at -18°C, the green beans energy value and available carbohydrates content were significantly increased by 24.09% and 48.28%, respectively, while, its moisture, ascorbic acid, sodium, potassium, calcium, magnesium, pH, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes were decreased by 1.60%, 3.46%, 16.30%, 3.02%, 9.13%, 5.19%, 20.93%, 35.89%, 36.33% and 31.81%, respectively as shown in Table (4.30). Figure (4.2) shows the effects of frozen storage on green beans minerals content.

Table (4.27): Effects of frozen storage on okra nutritional value and quality characteristics

Characteristics	Storage period in months						
	Zero time	2	4	6	8	10	12
	(n = 3 ± SD)						
Energy value (kcal/100g)	034.41 ± 0.04^{d}	035.97 ± 0.01^{c}	037.38 ± 0.05^{c}	038.86 ± 0.03^{b}	040.43 ± 0.04^{b}	041.66 ± 0.05^{a}	042.73 ± 0.02^{a}
Moisture %	086.32 ± 0.04^{b}	085.98 ± 0.02^{b}	085.67 ± 0.03^{d}	085.36 ± 0.06^{d}	085.01 ± 0.01^{c}	084.74 ± 0.04^{c}	084.52 ± 0.06^{a}
Protein %	001.32 ± 0.06^{c}	001.31 ± 0.03^{c}	001.29 ± 0.06^{b}	001.28 ± 0.01^{a}	001.27 ± 0.04^{a}	001.25 ± 0.05^{d}	001.24 ± 0.03^{d}
Fat %	000.35 ± 0.02^{d}	000.33 ± 0.02^{d}	000.32 ± 0.06^{c}	000.31 ± 0.05^{c}	000.29 ± 0.03^{b}	000.27 ± 0.02^{b}	000.25 ± 0.05^{a}
Total carbohydrates %	011.34 ± 0.01^{c}	011.75 ± 0.03^{b}	012.11 ± 0.04^{b}	012.47 ± 0.02^{b}	012.87 ± 0.05^{a}	013.21 ± 0.05^{a}	013.49 ± 0.06^{a}
Available carbohydrates %	006.40 ± 0.03^{a}	006.83 ± 0.01^{a}	007.21 ± 0.05^{b}	007.60 ± 0.03^{b}	008.03 ± 0.06^{c}	008.39 ± 0.01^{c}	008.70 ± 0.05^{c}
Crude fiber %	004.94 ± 0.03^{c}	004.92 ± 0.03^{b}	004.90 ± 0.02^{b}	004.87 ± 0.01^{b}	004.84 ± 0.01^{a}	004.82 ± 0.02^{a}	004.79 ± 0.03^{a}
Ash %	000.67 ± 0.01^{a}	000.63 ± 0.02^{a}	000.61 ± 0.03^{b}	000.58 ± 0.02^{b}	000.56 ± 0.06^{c}	000.53 ± 0.01^{c}	000.50 ± 0.04^{c}
Ascorbic acid (mg/100g)	026.57 ± 0.02^{d}	026.32 ± 0.04^{c}	026.14 ± 0.01^{c}	025.98 ± 0.01^{c}	025.72 ± 0.01^{b}	025.50 ± 0.02^{b}	025.35 ± 0.01^{a}
Sodium (mg/100g)	166.83 ± 0.05^{c}	159.15 ± 0.01^{b}	153.62 ± 0.03^{b}	148.26 ± 0.06^{b}	141.75 ± 0.06^{a}	134.85 ± 0.04^{a}	128.42 ± 0.04^{a}
Potassium (mg/100g)	656.37 ± 0.01^{d}	647.33 ± 0.05^{d}	638.27 ± 0.04^{d}	630.74 ± 0.07^{c}	622.47 ± 0.05^{c}	614.61 ± 0.03^{b}	602.63 ± 0.01^{a}
Calcium (mg/100g)	309.59 ± 0.06^{a}	301.77 ± 0.01^{a}	293.53 ± 0.01^{b}	284.32 ± 0.05^{b}	275.16 ± 0.03^{b}	264.72 ± 0.02^{c}	252.75 ± 0.03^{c}
Magnesium (mg/100g)	556.42 ± 0.03^{a}	547.45 ± 0.05^{b}	534.42 ± 0.03^{c}	521.45 ± 0.02^{c}	508.63 ± 0.02^{d}	495.43 ± 0.06^{d}	482.54 ± 0.04^{d}
pH	005.79 ± 0.01^{c}	005.53 ± 0.01^{c}	005.37 ± 0.06^{a}	005.10 ± 0.04^{c}	004.92 ± 0.02^{b}	004.75 ± 0.04^{c}	004.57 ± 0.01^{d}
Peroxidase activity %	009.32 ± 0.05^{a}	008.73 ± 0.03^{a}	008.09 ± 0.02^{b}	007.61 ± 0.05^{b}	006.94 ± 0.10^{c}	006.36 ± 0.06^{c}	005.80 ± 0.03^{c}
Lipoxygenase activity %	009.86 ± 0.03^{d}	009.22 ± 0.04^{c}	008.71 ± 0.01^{b}	008.08 ± 0.06^{b}	007.57 ± 0.03^{b}	007.22 ± 0.03^{a}	006.71 ± 0.02^{a}
Polyphenol oxidase activity %	009.81 ± 0.02^{d}	009.23 ± 0.06^{d}	008.69 ± 0.03^{c}	008.26 ± 0.02^{c}	007.88 ± 0.01^{b}	007.47 ± 0.02^{b}	006.98 ± 0.06^{a}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.28): Changes in okra energy value, chemical composition, pH and enzyme activity after frozen storage as percentage

Characteristics	Initial value	Changes as %
		After frozen storage
Energy value (kcal/100g)	034.41	+ 24.18
Moisture %	086.32	– 02.08
Available carbohydrates %	006.40	+ 35.94
Ascorbic acid (mg/100g)	026.57	– 04.59
Sodium (mg/100g)	166.83	– 23.02
Potassium (mg/100g)	656.37	– 08.19
Calcium (mg/100g)	309.59	– 18.36
Magnesium (mg/100g)	556.42	– 13.82
pH	005.79	– 21.07
Peroxidase activity %	009.32	– 37.77
Lipoxygenase activity %	009.86	– 31.95
Polyphenol oxidase activity %	009.81	– 28.85

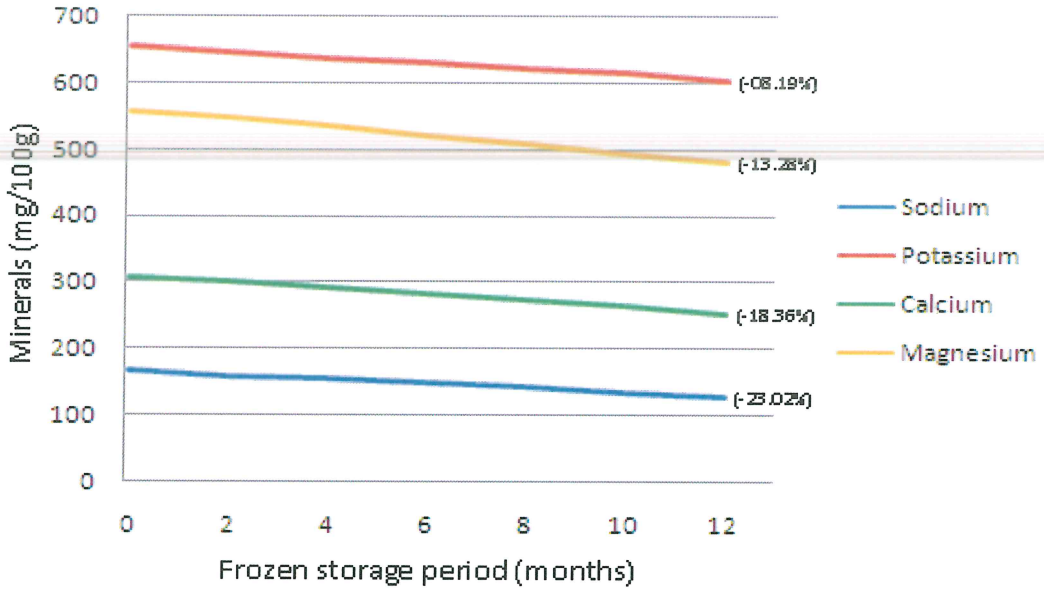

Figure (4.1): Effect of frozen storage on okra minerals content

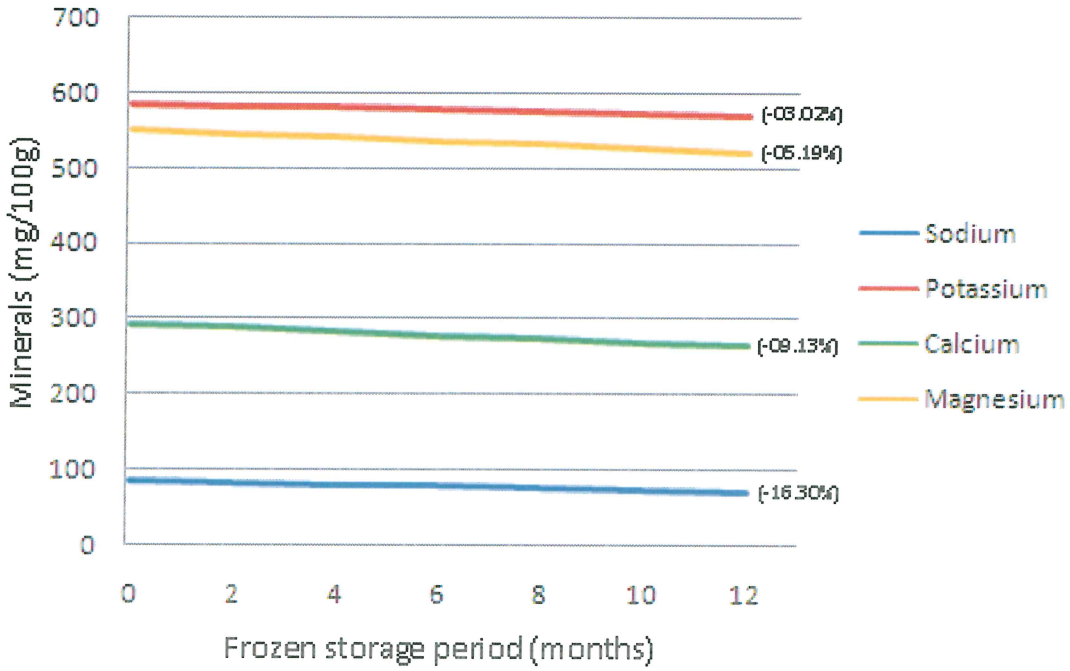

Figure (4.2): Effect of frozen storage on green beans minerals content

Table (4.29): Effects of frozen storage on green beans nutritional value and quality characteristics

Characteristics	Storage period in months						
	Zero time	2	4	6	8	10	12
	(n = 3 ± SD)						
Energy value (kcal/100g)	026.40 ± 0.06^{a}	027.43 ± 0.02^{a}	028.59 ± 0.05^{a}	029.74 ± 0.01^{b}	030.61 ± 0.03^{b}	031.69 ± 0.05^{c}	032.76 ± 0.01^{c}
Moisture %	087.68 ± 0.05^{d}	087.46 ± 0.02^{c}	087.21 ± 0.06^{c}	086.94 ± 0.03^{b}	086.76 ± 0.01^{b}	086.52 ± 0.04^{b}	086.28 ± 0.02^{a}
Protein %	001.72 ± 0.06^{a}	001.70 ± 0.04^{a}	001.67 ± 0.01^{b}	001.65 ± 0.05^{b}	001.63 ± 0.03^{b}	001.60 ± 0.06^{c}	001.58 ± 0.03^{c}
Fat %	000.36 ± 0.04^{a}	000.34 ± 0.06^{b}	000.32 ± 0.04^{b}	000.29 ± 0.03^{c}	000.27 ± 0.05^{c}	000.25 ± 0.02^{d}	000.22 ± 0.05^{d}
Total carbohydrates %	009.49 ± 0.05^{d}	009.77 ± 0.01^{d}	010.10 ± 0.03^{c}	010.44 ± 0.02^{b}	010.68 ± 0.04^{a}	010.99 ± 0.06^{a}	011.30 ± 0.01^{a}
Available carbohydrates %	004.06 ± 0.01^{a}	004.37 ± 0.06^{a}	004.72 ± 0.04^{a}	005.08 ± 0.01^{b}	005.35 ± 0.03^{c}	005.68 ± 0.02^{c}	006.02 ± 0.05^{c}
Crude fiber %	005.43 ± 0.02^{c}	005.40 ± 0.05^{c}	005.38 ± 0.03^{b}	005.36 ± 0.04^{b}	005.33 ± 0.01^{a}	005.31 ± 0.05^{a}	005.28 ± 0.02^{a}
Ash %	000.75 ± 0.01^{d}	000.73 ± 0.04^{c}	000.70 ± 0.06^{c}	000.68 ± 0.02^{b}	000.66 ± 0.05^{b}	000.64 ± 0.01^{a}	000.62 ± 0.03^{a}
Ascorbic acid (mg/100g)	038.68 ± 0.03^{a}	038.46 ± 0.05^{a}	038.22 ± 0.02^{b}	037.99 ± 0.04^{c}	037.74 ± 0.01^{d}	037.56 ± 0.06^{d}	037.34 ± 0.04^{d}
Sodium (mg/100g)	085.38 ± 0.04^{c}	082.36 ± 0.03^{c}	080.53 ± 0.05^{b}	078.73 ± 0.01^{a}	076.65 ± 0.06^{a}	073.58 ± 0.03^{a}	071.46 ± 0.02^{a}
Potassium (mg/100g)	586.53 ± 0.01^{a}	583.79 ± 0.04^{b}	581.67 ± 0.02^{b}	578.64 ± 0.05^{b}	575.57 ± 0.03^{c}	571.68 ± 0.01^{c}	568.83 ± 0.06^{d}
Calcium (mg/100g)	292.64 ± 0.02^{d}	288.82 ± 0.03^{c}	283.76 ± 0.05^{b}	278.47 ± 0.01^{b}	273.78 ± 0.04^{b}	270.03 ± 0.06^{a}	265.93 ± 0.05^{a}
Magnesium (mg/100g)	551.27 ± 0.03^{c}	546.89 ± 0.01^{c}	542.36 ± 0.05^{c}	537.69 ± 0.02^{b}	533.24 ± 0.04^{a}	527.82 ± 0.05^{a}	522.64 ± 0.03^{a}
pH	006.64 ± 0.06^{a}	006.39 ± 0.03^{a}	006.15 ± 0.05^{a}	005.92 ± 0.02^{b}	005.68 ± 0.04^{b}	005.41 ± 0.06^{b}	005.25 ± 0.01^{c}
Peroxidase activity %	009.00 ± 0.06^{d}	008.47 ± 0.04^{c}	007.83 ± 0.01^{c}	007.31 ± 0.05^{b}	006.62 ± 0.02^{b}	006.02 ± 0.04^{a}	005.77 ± 0.03^{a}
Lipoxygenase activity %	009.55 ± 0.04^{a}	008.86 ± 0.01^{a}	008.35 ± 0.05^{b}	007.73 ± 0.03^{b}	007.14 ± 0.06^{c}	006.63 ± 0.02^{c}	006.08 ± 0.04^{c}
Polyphenol oxidase activity %	009.62 ± 0.01^{c}	008.98 ± 0.05^{c}	008.49 ± 0.03^{b}	007.97 ± 0.06^{b}	007.49 ± 0.04^{a}	007.04 ± 0.03^{a}	006.56 ± 0.01^{a}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.30): Changes in green beans energy value, chemical composition, pH and enzyme activity after frozen storage as percentage

Characteristics	Initial value	Changes as %
		After frozen storage
Energy value (kcal/100g)	026.40	+ 24.09
Moisture %	087.68	– 01.60
Available carbohydrates %	004.06	+ 48.28
Ascorbic acid (mg/100g)	038.68	– 03.46
Sodium (mg/100g)	085.38	– 16.30
Potassium (mg/100g)	586.53	– 03.02
Calcium (mg/100g)	292.64	– 09.13
Magnesium (mg/100g)	551.27	– 05.19
pH	006.64	– 20.93
Peroxidase activity %	009.00	– 35.89
Lipoxygenase activity %	009.55	– 36.33
Polyphenol oxidase activity %	009.62	– 31.81

The results obtained in this study are in agreement with those stated by **Puupponen- Pimiä *et al.* (2003); Sahari *et al.* (2004)** and **Matkowski (2009). Favell (1998)** found that, after a storage period of 12 months at -20°C, the decrease in the ascorbic acid content of some vegetables was less than 20%.

4.6.3 Peas

Table (4.31) shows the effects of frozen storage on peas nutritional value, pH and enzymatic activity. The results indicate that, after a frozen storage period of 12 months at -18°C, the peas energy value and its available carbohydrates content significantly increased by 19.75% and 34.98%, respectively while, its moisture, ascorbic acid, sodium, potassium, calcium, magnesium, pH, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes were decreased by 1.90%, 3.70%, 9.80%, 4.67%, 10.05%, 6.92%, 20.62%, 33.33%, 40.58% and 36.13%, respectively (Table, 4.32). Figure (4.3) indicates the effects of frozen storage on peas minerals content. The results obtained in this study are in accordance with those reported by **Puupponen- Pimiä *et al.* (2003)** and **Sahari *et al.* (2004)**.

4.6.4 Spinach

Table (4.33) presents the effects of frozen storage on spinach nutritional value, pH and enzymatic activity. The results show that, after a frozen storage period of 12 months at -18°C, the spinach energy value and available carbohydrates content significantly increased by 18.56% and 29.90%, respectively due to the gradual decrease in its moisture content. While, its ascorbic acid, sodium, potassium, calcium, magnesium, pH, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes decreased by 3.46%, 8.12%, 3.42%, 8.60%, 6.64%, 21.06%, 40.79%, 40.98% and 37.20%, respectively as illustrated in Table (4.34). The decrease in spinach pH, ascorbic acid and minerals

Table (4.31): Effects of frozen storage on peas nutritional value and quality characteristics

Characteristics	Storage period in months						
	Zero time	2	4	6	8	10	12
	(n = 3 ± SD)						
Energy value (kcal/100g)	036.96 ± 0.01^{d}	037.99 ± 0.05^{d}	039.15 ± 0.02^{c}	040.64 ± 0.04^{c}	041.82 ± 0.06^{b}	043.06 ± 0.05^{a}	044.26 ± 0.01^{a}
Moisture %	085.36 ± 0.04^{a}	085.13 ± 0.01^{a}	084.87 ± 0.05^{a}	084.55 ± 0.03^{b}	084.28 ± 0.06^{b}	084.00 ± 0.04^{c}	083.74 ± 0.02^{c}
Protein %	001.83 ± 0.01^{a}	001.81 ± 0.04^{b}	001.78 ± 0.02^{b}	001.76 ± 0.06^{c}	001.64 ± 0.03^{c}	001.61 ± 0.05^{d}	001.59 ± 0.01^{d}
Fat %	000.39 ± 0.04^{c}	000.37 ± 0.02^{c}	000.35 ± 0.05^{c}	000.33 ± 0.01^{b}	000.30 ± 0.06^{b}	000.28 ± 0.03^{b}	000.26 ± 0.05^{a}
Total carbohydrates %	011.78 ± 0.02^{a}	012.07 ± 0.05^{a}	012.40 ± 0.03^{a}	012.79 ± 0.06^{b}	013.23 ± 0.03^{c}	013.58 ± 0.04^{c}	013.90 ± 0.01^{d}
Available carbohydrates %	006.46 ± 0.04^{d}	006.77 ± 0.02^{d}	007.12 ± 0.01^{c}	007.54 ± 0.04^{c}	008.00 ± 0.06^{b}	008.37 ± 0.05^{a}	008.72 ± 0.03^{a}
Crude fiber %	005.32 ± 0.01^{d}	005.30 ± 0.04^{c}	005.28 ± 0.06^{c}	005.25 ± 0.03^{b}	005.23 ± 0.05^{b}	005.21 ± 0.02^{b}	005.18 ± 0.05^{a}
Ash %	000.64 ± 1.06^{a}	000.62 ± 0.01^{a}	000.60 ± 0.04^{b}	000.57 ± 0.02^{b}	000.55 ± 0.05^{c}	000.53 ± 0.03^{d}	000.51 ± 0.02^{d}
Ascorbic acid (mg/100g)	037.53 ± 0.05^{a}	037.35 ± 0.02^{a}	037.12 ± 0.04^{b}	036.84 ± 0.06^{b}	036.61 ± 0.03^{b}	036.37 ± 0.01^{c}	036.14 ± 0.05^{c}
Sodium (mg/100g)	174.64 ± 0.04^{d}	171.46 ± 0.03^{c}	168.73 ± 0.05^{c}	165.85 ± 0.01^{b}	163.28 ± 0.02^{a}	160.63 ± 0.06^{a}	157.52 ± 0.04^{a}
Potassium (mg/100g)	418.39 ± 0.03^{d}	414.86 ± 0.05^{c}	411.64 ± 0.01^{c}	408.78 ± 0.06^{c}	405.72 ± 0.02^{b}	401.92 ± 0.03^{b}	398.86 ± 0.02^{a}
Calcium (mg/100g)	301.83 ± 0.05^{a}	295.97 ± 0.01^{a}	291.74 ± 0.04^{b}	286.88 ± 0.02^{b}	280.79 ± 0.06^{c}	275.61 ± 0.03^{c}	271.48 ± 0.01^{d}
Magnesium (mg/100g)	474.72 ± 0.01^{a}	468.47 ± 0.06^{a}	463.78 ± 0.03^{b}	456.91 ± 0.04^{b}	452.73 ± 0.05^{c}	448.26 ± 0.02^{c}	441.89 ± 0.06^{d}
pH	006.79 ± 0.02^{c}	006.54 ± 0.03^{c}	006.31 ± 0.05^{b}	006.05 ± 0.01^{b}	005.81 ± 0.06^{a}	005.59 ± 0.02^{a}	005.39 ± 0.04^{a}
Peroxidase activity %	009.84 ± 0.05^{d}	009.37 ± 0.04^{d}	008.78 ± 0.02^{c}	008.21 ± 0.05^{c}	007.68 ± 0.01^{b}	007.07 ± 0.06^{b}	006.56 ± 0.03^{a}
Lipoxygenase activity %	009.29 ± 0.03^{a}	008.57 ± 0.01^{a}	007.93 ± 0.05^{a}	007.37 ± 0.04^{b}	006.74 ± 0.02^{b}	006.13 ± 0.04^{c}	005.52 ± 0.06^{d}
Polyphenol oxidase activity %	009.16 ± 0.02^{a}	008.56 ± 0.05^{a}	007.95 ± 0.03^{b}	007.47 ± 0.02^{b}	006.95 ± 0.04^{b}	006.41 ± 0.06^{c}	005.85 ± 0.03^{c}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.32): Changes in peas energy value, chemical composition, pH and enzyme activity after frozen storage as percentage

Characteristics	Initial value	Changes as %
		After frozen storage
Energy value (kcal/100g)	036.96	+ 19.75
Moisture %	085.36	– 01.90
Available carbohydrates %	006.46	+ 34.98
Ascorbic acid (mg/100g)	037.53	– 03.70
Sodium (mg/100g)	174.64	– 09.80
Potassium (mg/100g)	418.39	– 04.67
Calcium (mg/100g)	301.83	– 10.05
Magnesium (mg/100g)	474.72	– 06.92
pH	006.79	– 20.62
Peroxidase activity %	009.84	– 33.33
Lipoxygenase activity %	009.29	– 40.58
Polyphenol oxidase activity %	009.16	– 36.13

Table (4.33): Effects of frozen storage on spinach nutritional value and quality characteristics

Characteristics	Storage period in months						
	Zero time	2	4	6	8	10	12
	(n = 3 ± SD)						
Energy value (kcal/100g)	040.31 ± 0.05^{c}	040.80 ± 0.03^{c}	043.00 ± 0.01^{c}	044.24 ± 0.02^{b}	045.27 ± 0.06^{a}	046.51 ± 0.04^{a}	047.79 ± 0.01^{a}
Moisture %	084.69 ± 0.04^{a}	084.45 ± 0.02^{a}	084.19 ± 0.05^{b}	083.91 ± 0.01^{b}	083.68 ± 0.03^{b}	083.42 ± 0.04^{c}	083.14 ± 0.06^{d}
Protein %	001.62 ± 0.01^{a}	001.59 ± 0.06^{b}	001.57 ± 0.04^{b}	001.55 ± 0.05^{c}	001.53 ± 0.05^{c}	001.50 ± 0.02^{d}	001.48 ± 0.01^{d}
Fat %	000.43 ± 0.02^{c}	000.41 ± 0.04^{c}	000.39 ± 0.02^{b}	000.36 ± 0.03^{b}	000.34 ± 0.01^{a}	000.32 ± 0.06^{a}	000.30 ± 0.03^{a}
Total carbohydrates %	012.52 ± 0.01^{a}	012.83 ± 0.05^{a}	013.15 ± 0.03^{b}	013.51 ± 0.04^{b}	013.80 ± 0.02^{c}	014.14 ± 0.01^{c}	014.49 ± 0.06^{d}
Available carbohydrates %	007.39 ± 0.02^{a}	007.82 ± 0.02^{a}	008.17 ± 0.01^{a}	008.55 ± 0.06^{b}	008.86 ± 0.03^{b}	009.23 ± 0.05^{c}	009.60 ± 0.02^{c}
Crude fiber %	005.03 ± 0.03^{d}	005.01 ± 0.06^{c}	004.98 ± 0.05^{b}	004.96 ± 0.02^{b}	004.94 ± 0.04^{b}	004.91 ± 0.03^{a}	004.89 ± 0.01^{a}
Ash %	000.74 ± 0.01^{a}	000.72 ± 0.05^{b}	000.70 ± 0.06^{b}	000.67 ± 0.03^{c}	000.65 ± 0.02^{c}	000.62 ± 0.02^{d}	000.59 ± 0.04^{d}
Ascorbic acid (mg/100g)	044.16 ± 0.04^{a}	043.82 ± 0.02^{a}	043.58 ± 0.04^{a}	043.35 ± 0.05^{b}	043.11 ± 0.03^{b}	042.87 ± 0.01^{c}	042.63 ± 0.03^{d}
Sodium (mg/100g)	211.84 ± 0.03^{d}	208.65 ± 0.05^{d}	205.72 ± 0.02^{c}	202.81 ± 0.06^{c}	198.93 ± 0.01^{b}	196.88 ± 0.03^{b}	194.63 ± 0.05^{a}
Potassium (mg/100g)	605.31 ± 0.04^{a}	601.62 ± 0.06^{a}	597.77 ± 0.01^{b}	594.61 ± 0.03^{b}	590.96 ± 0.04^{c}	587.75 ± 0.02^{c}	584.61 ± 0.06^{c}
Calcium (mg/100g)	403.59 ± 0.02^{a}	398.42 ± 0.03^{a}	392.68 ± 0.05^{a}	386.54 ± 0.01^{b}	380.75 ± 0.04^{b}	374.53 ± 0.03^{c}	368.86 ± 0.02^{d}
Magnesium (mg/100g)	578.72 ± 0.04^{d}	571.65 ± 0.06^{d}	564.51 ± 0.02^{c}	558.78 ± 0.05^{c}	552.62 ± 0.03^{b}	546.47 ± 0.06^{b}	540.28 ± 0.04^{a}
pH	006.98 ± 0.02^{a}	006.72 ± 0.04^{a}	006.48 ± 0.06^{a}	006.21 ± 0.01^{b}	005.96 ± 0.05^{b}	005.77 ± 0.03^{c}	005.51 ± 0.05^{d}
Peroxidase activity %	008.58 ± 0.03^{a}	008.04 ± 0.05^{a}	007.40 ± 0.02^{b}	006.87 ± 0.06^{b}	006.23 ± 0.03^{c}	005.63 ± 0.04^{d}	005.08 ± 0.01^{d}
Lipoxygenase activity %	008.76 ± 0.05^{d}	008.08 ± 0.04^{d}	007.46 ± 0.01^{c}	006.88 ± 0.03^{c}	006.25 ± 0.06^{b}	005.71 ± 0.05^{a}	005.17 ± 0.03^{a}
Polyphenol oxidase activity %	008.87 ± 0.02^{a}	008.22 ± 0.01^{b}	007.63 ± 0.05^{b}	007.15 ± 0.03^{b}	006.68 ± 0.05^{c}	006.14 ± 0.02^{d}	005.57 ± 0.04^{d}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.34): Changes in spinach energy value, chemical composition, pH and enzyme activity after frozen storage as percentage

Characteristics	Initial value	Changes as %
		After frozen storage
Energy value (kcal/100g)	040.31	+ 18.56
Moisture %	084.69	– 01.83
Available carbohydrates %	007.39	+ 29.90
Ascorbic acid (mg/100g)	044.16	– 03.46
Sodium (mg/100g)	211.84	– 08.12
Potassium (mg/100g)	605.31	– 03.42
Calcium (mg/100g)	403.59	– 08.60
Magnesium (mg/100g)	578.72	– 06.64
pH	006.98	– 21.06
Peroxidase activity %	008.58	– 40.79
Lipoxygenase activity %	008.76	– 40.98
Polyphenol oxidase activity %	008.87	– 37.20

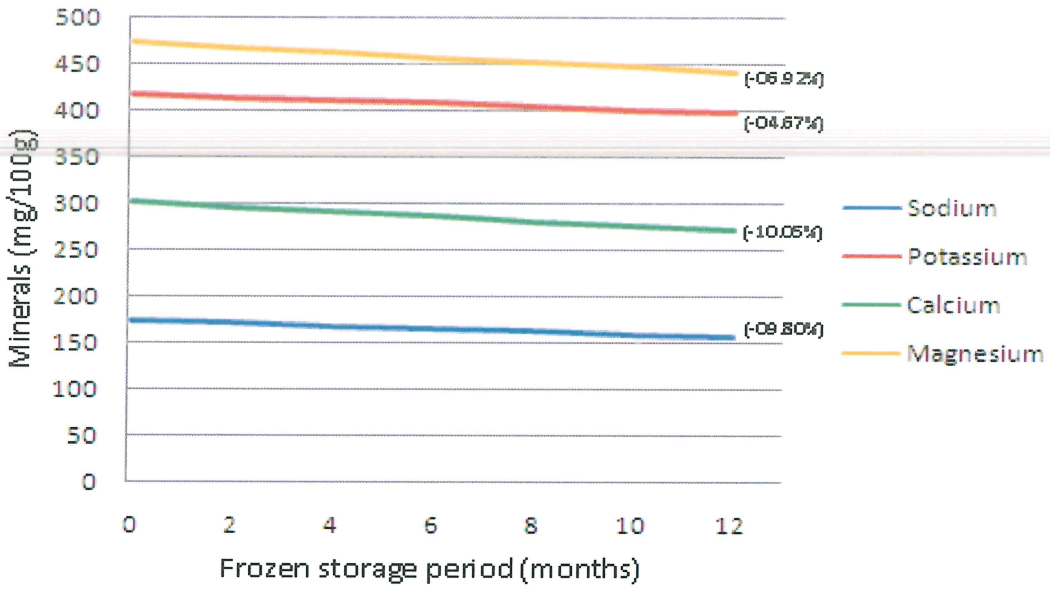

Figure (4.3): Effect of frozen storage on peas minerals content

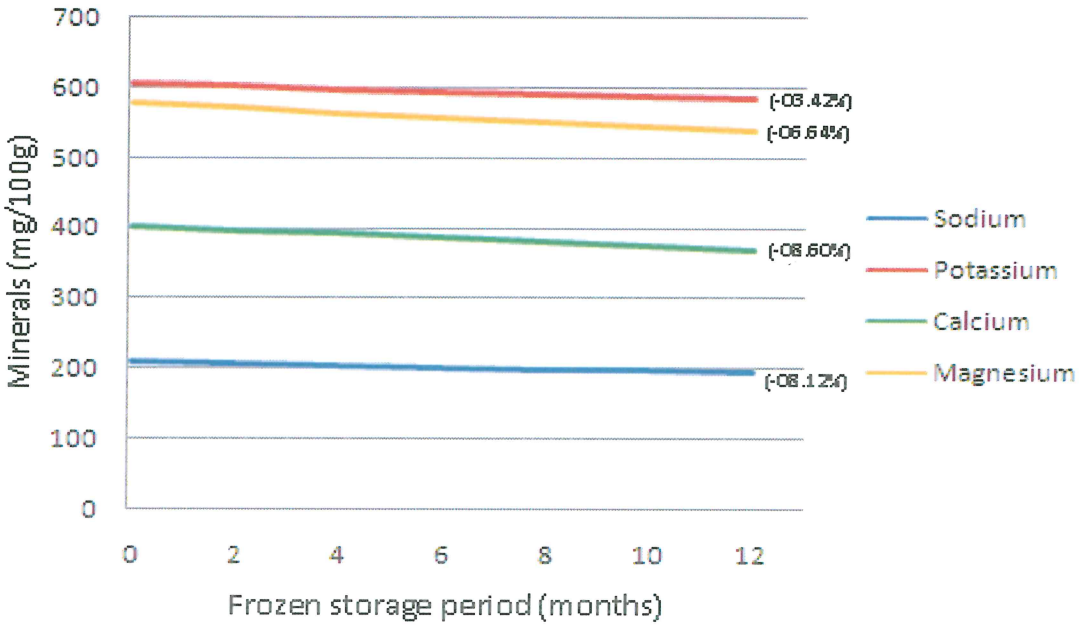

Figure (4.4): Effect of frozen storage on spinach minerals content

content may be due to the increment of its available carbohydrates during the storage period.

However, the effects of frozen storage on spinach minerals content are presented in Figure (4.4). The results obtained in this study agree well with those reported by **Sahari *et al.* (2004)** and **Matkowski (2009)**. The vitamin losses during the frozen storage was found to depend mainly on products storage temperature, type of packaging material and utilization of food additives **(Garden-Robinson, 2013)**.

4.6.5 Molokhia

Table (4.35) and (4.36) shows the effects of frozen storage on molokhia nutritional value, pH and enzymatic activity. From the results obtained in this study the energy value and the available carbohydrates content in molokhia vegetable significantly increased after storage period of 12 months at -18°C by 20.54% and 31.14%, respectively. While, the moisture, ascorbic acid, sodium, potassium, calcium, magnesium, pH, the activities of peroxidase, lipoxygenase and polyphenol oxidase enzymes decreased by 2.14%, 4.83%, 17.63%, 2.91%, 17.56%, 5.70%, 20.74%, 35.74%, 42.96% and 34.75%, respectively. Figure (4.5) shows the effects of frozen storage on molokhia minerals content. The results obtained in this study are in a good agreement with those mentioned by **Puupponen- Pimiä *et al.* (2003)** and **Matkowski (2009)**. **Czarnowska** and **Gujska (2012)** stated that, storage of frozen vegetables for a long period (12 months) cause marked losses and effects on their vitamins, colours and flavours.

In general, the effects of frozen storage on vegetables energy value, moisture, available carbohydrates and ascorbic acid contents are indicated in Figures (4.6), (4.7), (4.8) and (4.9), respectively. Whereas, the effects of frozen storage on vegetables pH, peroxidase, lipoxygenase and polyphenol oxidase activities are shown in figures (4.10), (4.11), (4.12) and (4.13), respectively.

Table (4.35): Effects of frozen storage on molokhia nutritional value and quality characteristics

Characteristics	Storage period in months						
	Zero time	2	4	6	8	10	12
	(n = 3 ± SD)						
Energy value (kcal/100g)	039.77 ± 0.03^{a}	041.13 ± 0.02^{a}	042.66 ± 0.05^{b}	044.02 ± 0.01^{b}	045.22 ± 0.06^{c}	046.50 ± 0.01^{c}	047.94 ± 0.04^{d}
Moisture %	085.90 ± 0.02^{c}	085.58 ± 0.06^{c}	085.24 ± 0.04^{c}	084.93 ± 0.03^{b}	084.66 ± 0.06^{a}	084.38 ± 0.02^{a}	084.06 ± 0.05^{a}
Protein %	001.18 ± 0.03^{d}	001.16 ± 0.05^{d}	001.14 ± 0.02^{c}	001.12 ± 0.01^{c}	001.10 ± 0.04^{b}	001.07 ± 0.05^{b}	001.05 ± 0.02^{a}
Fat %	000.46 ± 0.04^{a}	000.43 ± 0.02^{a}	000.41 ± 0.06^{a}	000.39 ± 0.02^{b}	000.37 ± 0.05^{b}	000.35 ± 0.01^{c}	000.33 ± 0.03^{c}
Total carbohydrates %	011.73 ± 0.03^{a}	012.12 ± 0.05^{b}	012.52 ± 0.02^{b}	012.89 ± 0.04^{b}	013.22 ± 0.01^{c}	013.58 ± 0.06^{d}	013.96 ± 0.04^{d}
Available carbohydrates %	007.61 ± 0.05^{d}	008.02 ± 0.02^{d}	008.45 ± 0.05^{c}	008.84 ± 0.03^{c}	009.19 ± 0.06^{b}	009.57 ± 0.01^{b}	009.98 ± 0.03^{a}
Crude fiber %	004.12 ± 0.06^{a}	004.10 ± 0.04^{a}	004.07 ± 0.03^{a}	004.05 ± 0.02^{b}	004.03 ± 0.05^{c}	004.01 ± 0.03^{c}	003.98 ± 0.06^{c}
Ash %	000.73 ± 0.01^{a}	000.71 ± 0.04^{b}	000.69 ± 0.02^{b}	000.67 ± 0.05^{c}	000.65 ± 0.03^{c}	000.62 ± 0.06^{d}	000.60 ± 0.01^{d}
Ascorbic acid (mg/100g)	034.60 ± 0.02^{d}	034.32 ± 0.05^{d}	034.06 ± 0.03^{c}	033.81 ± 0.04^{c}	033.56 ± 0.01^{b}	033.22 ± 0.03^{b}	032.93 ± 0.02^{a}
Sodium (mg/100g)	106.32 ± 0.04^{a}	103.65 ± 0.03^{a}	100.83 ± 0.05^{b}	097.76 ± 0.04^{b}	093.92 ± 0.02^{b}	090.47 ± 0.04^{c}	087.58 ± 0.05^{c}
Potassium (mg/100g)	711.64 ± 0.03^{a}	708.53 ± 0.06^{a}	704.89 ± 0.02^{a}	701.74 ± 0.03^{b}	697.54 ± 0.06^{c}	694.72 ± 0.02^{d}	690.94 ± 0.04^{d}
Calcium (mg/100g)	171.85 ± 0.03^{b}	166.57 ± 0.04^{b}	160.96 ± 0.03^{d}	155.78 ± 0.05^{a}	151.35 ± 0.01^{d}	146.47 ± 0.06^{b}	141.68 ± 0.03^{d}
Magnesium (mg/100g)	604.24 ± 0.04^{d}	597.79 ± 0.05^{c}	591.68 ± 0.02^{b}	586.61 ± 0.06^{b}	580.73 ± 0.04^{b}	575.32 ± 0.03^{a}	569.78 ± 0.05^{a}
pH	007.33 ± 0.01^{d}	007.09 ± 0.03^{d}	006.83 ± 0.06^{c}	006.57 ± 0.02^{c}	006.31 ± 0.03^{b}	006.07 ± 0.05^{b}	005.81 ± 0.01^{a}
Peroxidase activity %	009.57 ± 0.04^{a}	009.04 ± 0.01^{b}	008.51 ± 0.05^{b}	008.05 ± 0.04^{c}	007.36 ± 0.05^{c}	006.73 ± 0.02^{d}	006.15 ± 0.06^{d}
Lipoxygenase activity %	008.38 ± 0.05^{c}	007.75 ± 0.05^{c}	007.12 ± 0.02^{b}	006.56 ± 0.06^{b}	005.96 ± 0.01^{a}	005.38 ± 0.04^{a}	004.78 ± 0.02^{a}
Polyphenol oxidase activity %	009.38 ± 0.01^{a}	008.76 ± 0.03^{a}	008.24 ± 0.04^{b}	007.73 ± 0.03^{b}	007.21 ± 0.02^{c}	006.68 ± 0.06^{c}	006.12 ± 0.01^{d}

n = number of independent determinations.
SD = standard deviation.
Mean value(s) ± SD having different superscript letter(s) in each row are significantly different ($P \leq 0.05$).

Table (4.36): Changes in molokhia energy value, chemical composition, pH and enzyme activity after frozen storage as percentage

Characteristics	Initial value	Changes as %
		After frozen storage
Energy value (kcal/100g)	039.77	+ 20.54
Moisture %	085.90	– 02.14
Available carbohydrates %	007.61	+ 31.14
Ascorbic acid (mg/100g)	034.60	– 04.83
Sodium (mg/100g)	106.32	– 17.63
Potassium (mg/100g)	711.64	– 02.91
Calcium (mg/100g)	171.85	– 17.56
Magnesium (mg/100g)	604.24	– 05.70
pH	007.33	– 20.74
Peroxidase activity %	009.57	– 35.74
Lipoxygenase activity %	008.38	– 42.96
Polyphenol oxidase activity %	009.38	– 34.75

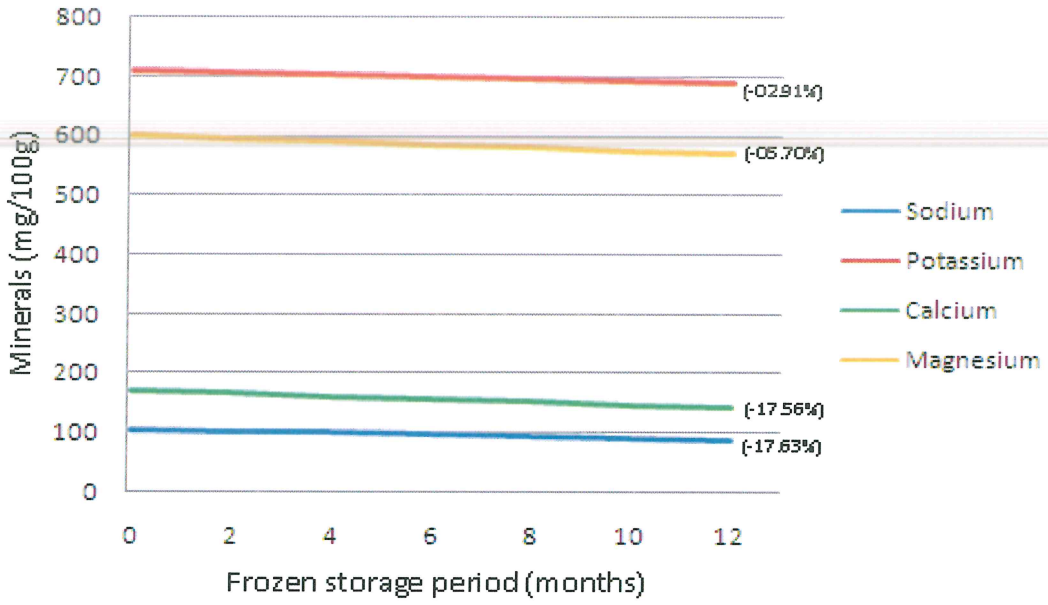

Figure (4.5): Effect of frozen storage on molokhia minerals content

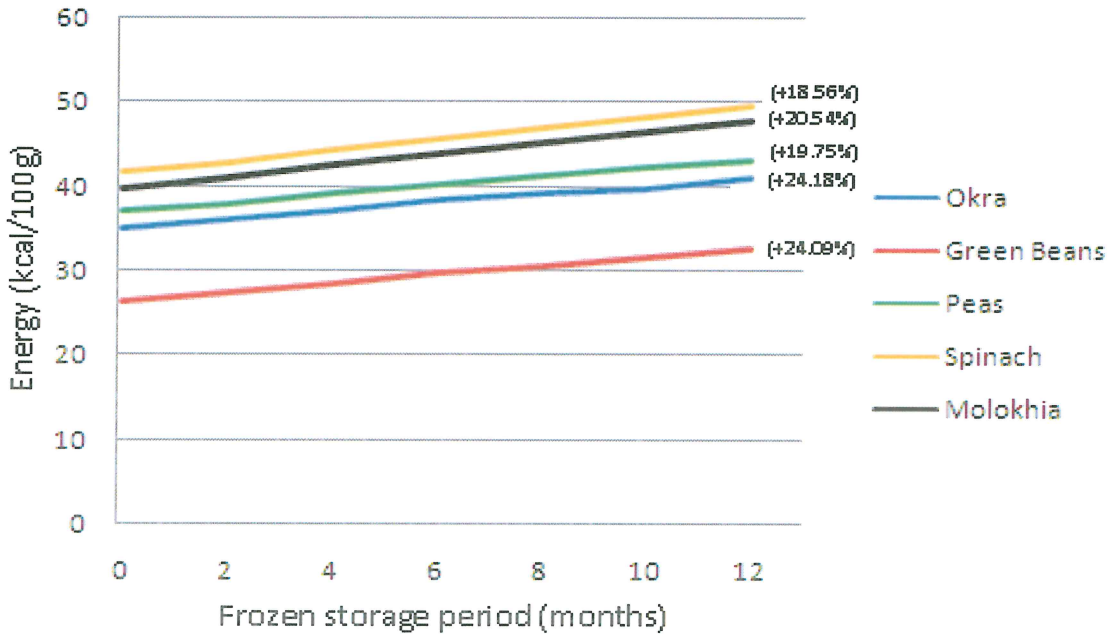

Figure (4.6): Effect of frozen storage on vegetables energy content

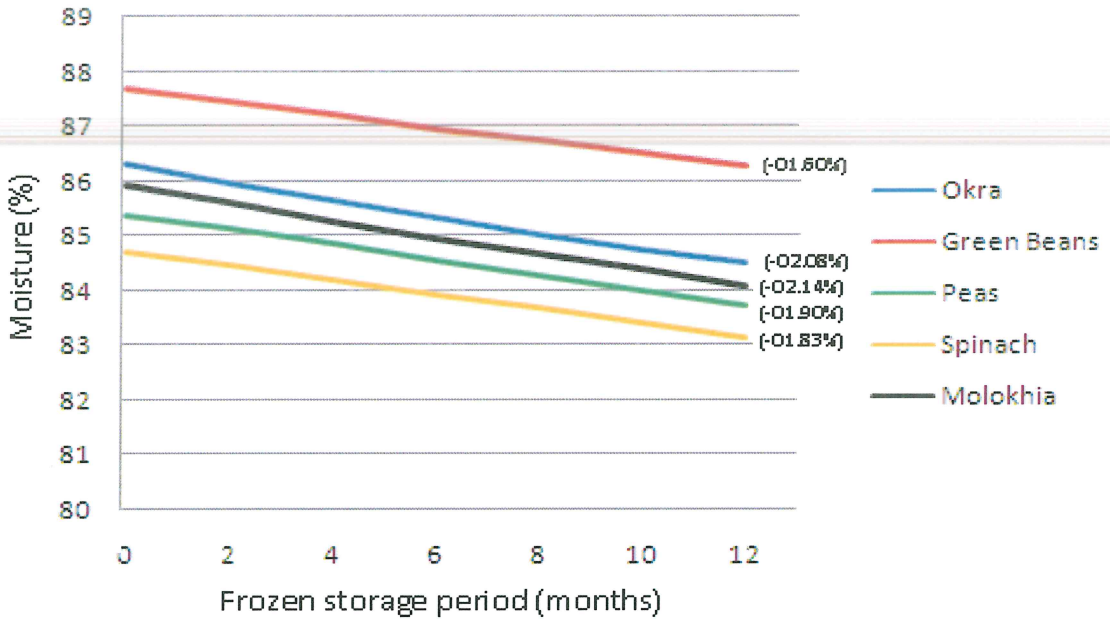

Figure (4.7): Effect of frozen storage on vegetables moisture content

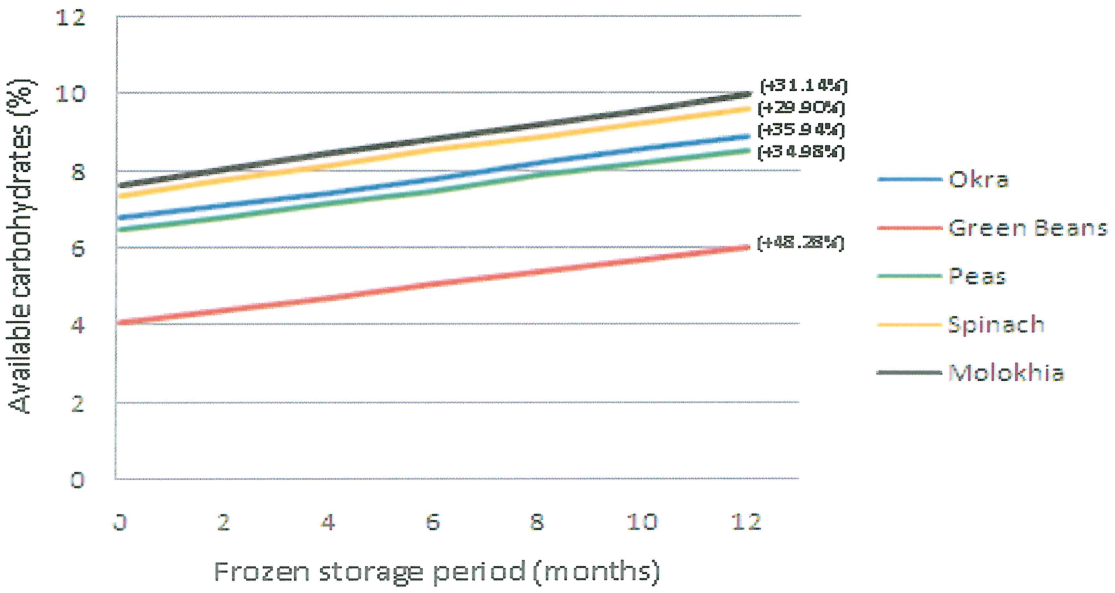

Figure (4.8): Effect of frozen storage on vegetables available carbohydrates content

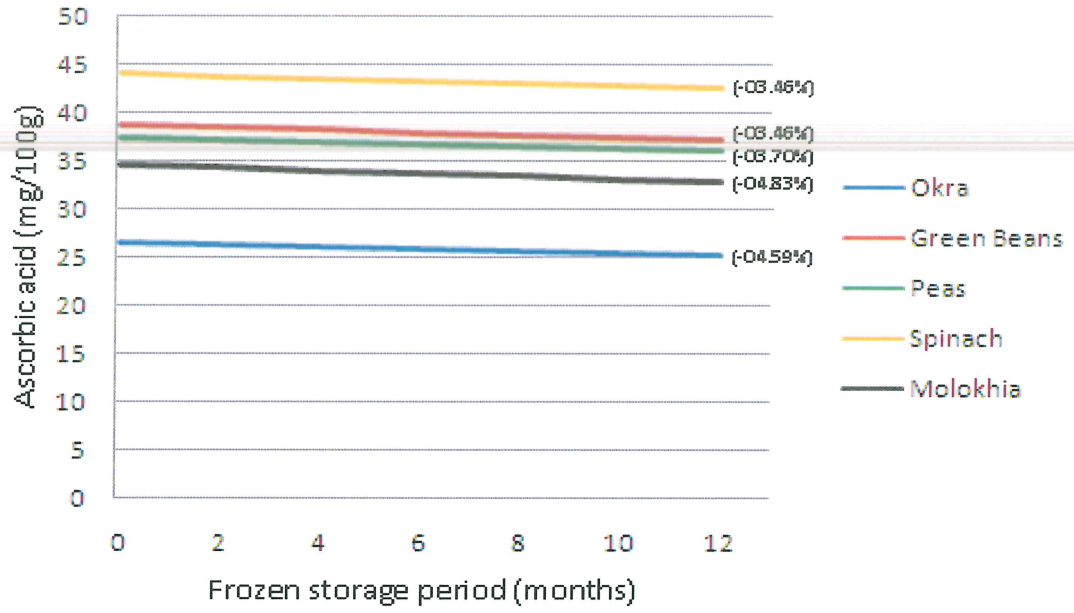

Figure (4.9): Effect of frozen storage on vegetables ascorbic acid content

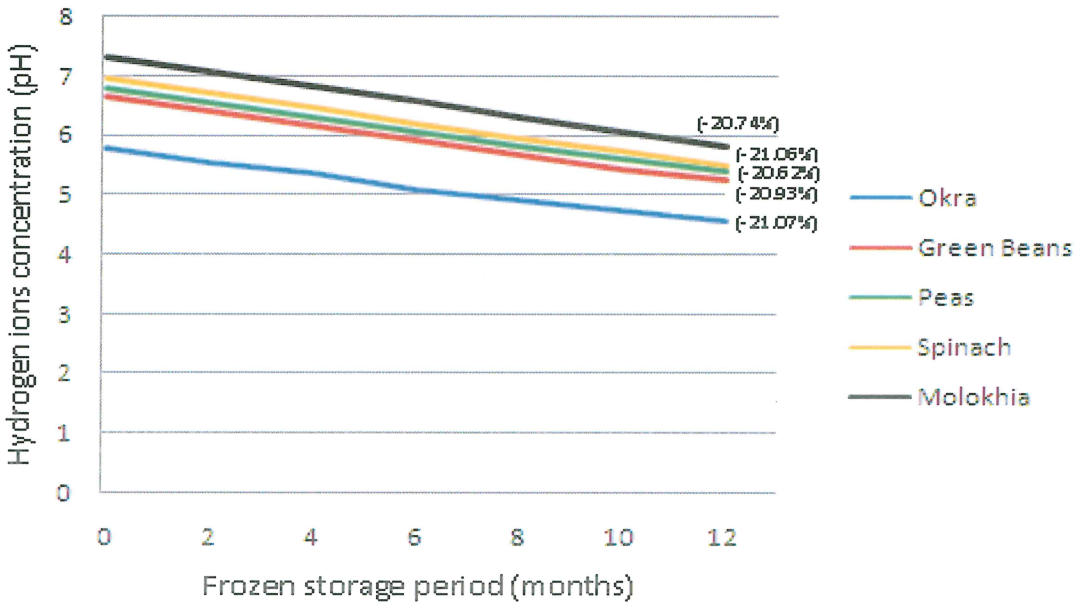

Figure (4.10): Effect of frozen storage on vegetables hydrogen ions concentration (pH)

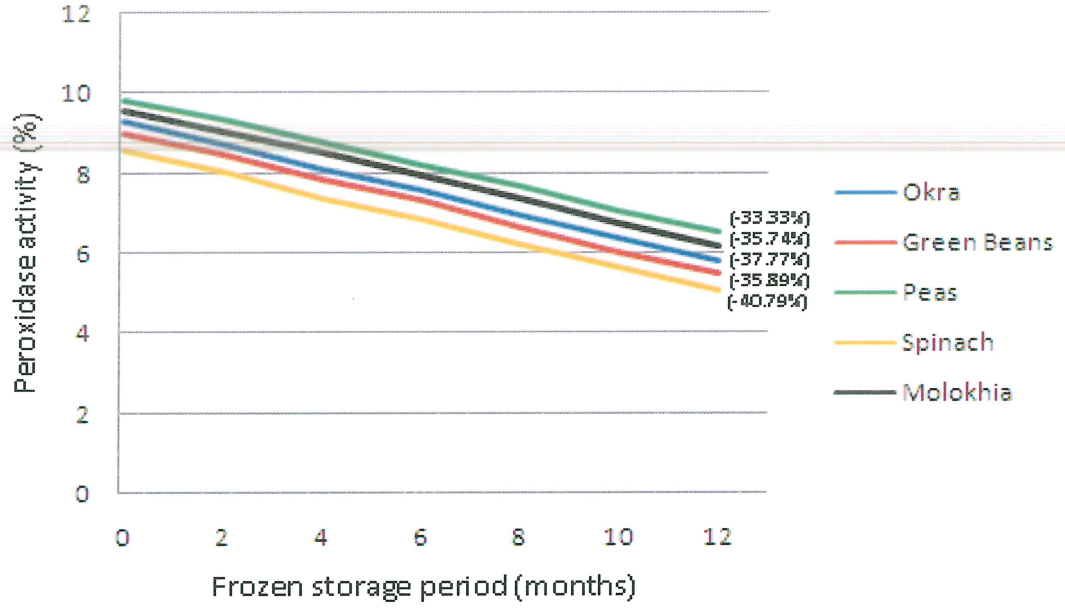

Figure (4.11): Effect of frozen storage on vegetables peroxidase activity

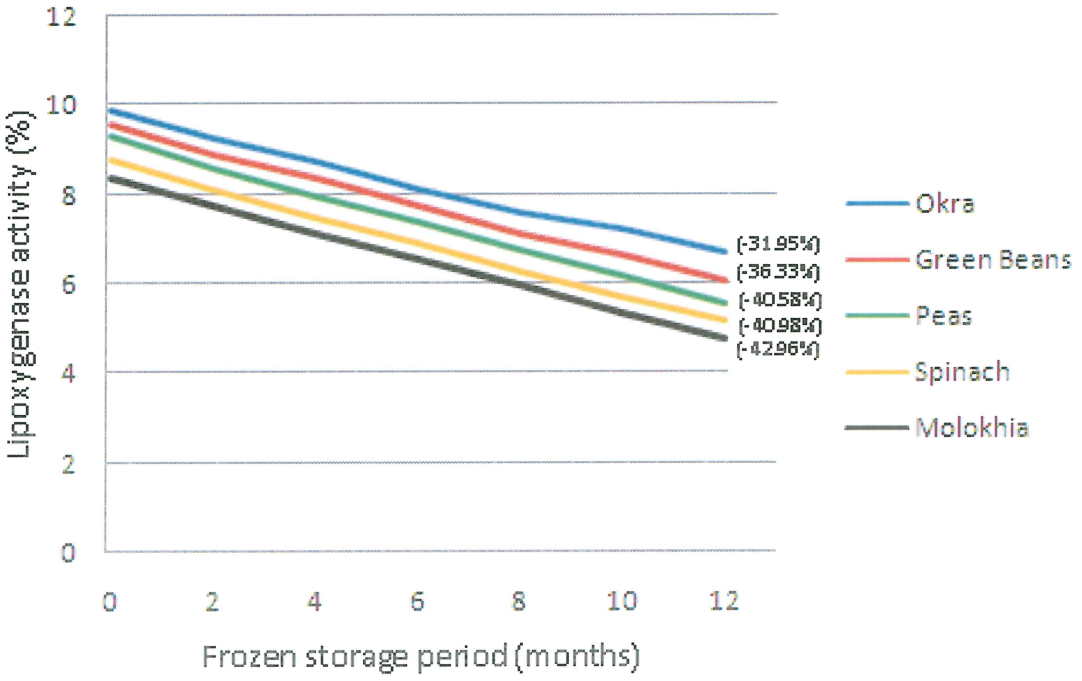

Figure (4.12): Effect of frozen storage on vegetables lipoxygenase activity

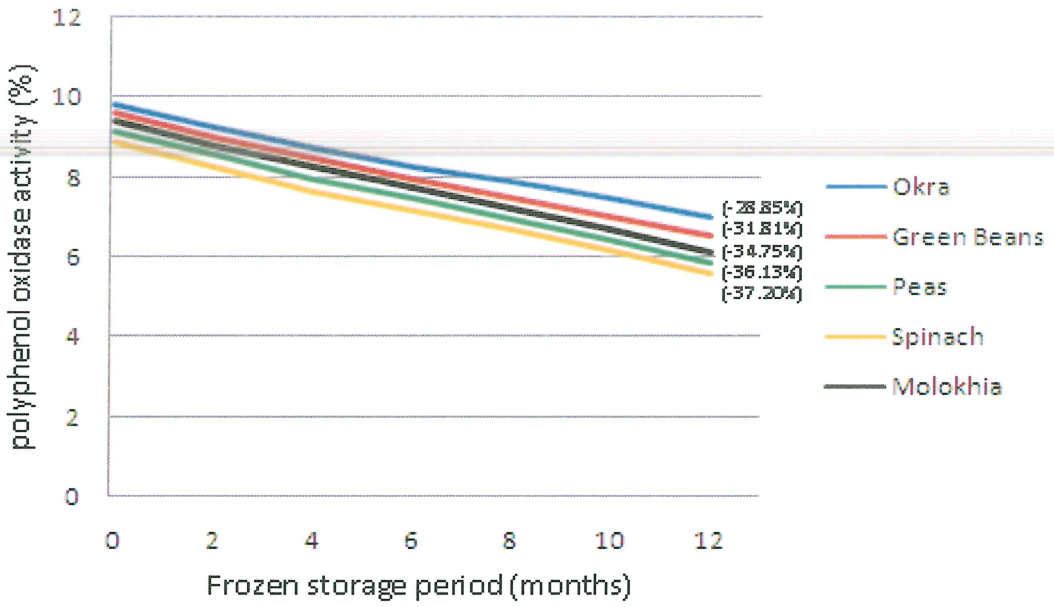

Figure (4.13): Effect of frozen storage on vegetables polyphenol oxidase activity

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the results obtained in this study it can be concluded that, fresh vegetables namely okra, green beans, peas, spinach and molokhia are found with high moisture content, very rich in fibers, ascorbic acid, sodium, potassium, calcium, magnesium and with low calories, protein and fat contents. Also, the aforesaid vegetables are of high peroxidase, lipoxygenase and polyphenol oxidase activities.

During the freezing process, the blanching step greatly reduced the levels of enzymes activities, ascorbic acid and minerals contents with slight increment in moisture and available carbohydrates levels. After the freezing step, the energy values and available carbohydrates contents in all vegetables slightly increased with a slight decrease in their moisture content, ascorbic acid, minerals, pH and enzymes activity in comparison with their values after the blanching step.

Also, the same previous trends during the freezing step were observed after frozen storage period of 12 months at -18°C in all vegetables. Moreover, the energy values and available carbohydrates in the different vegetables during the frozen storage markedly increased with slight decrease in their moisture, ascorbic acid, minerals, pH and enzymes activity in comparison with their values after the freezing step.

5.2 Recommendations

1. Vegetables should be immediately frozen after harvesting.
2. During freezing of fresh vegetables, steam blanching at high temperature - short time (HTST) as well as individual quick freezing (IQF) method should be used.

3. Vegetables could be stored with high nutritional values at -18°C for 12 months.
4. Additional investigations are definitely needed to determine the effects of the freezing process and frozen storage on vegetables natural pigments, other vitamins and microbial activity.
5. Also, the effects of the frozen storage and types of packaging materials on vegetables nutritional value should be investigated.
6. Additional studies are also needed to determine market demands and feasibility of frozen vegetable products at local and international markets.
7. The investment in the field of food freezing industry should be encouraged by government through offering considerable and valuable incentives and facilities.

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APPENDICES

APPENDICES

Blancher of vegetables processing line

Freezer of vegetables processing line

Blancher of Vegetable leaves processing line

Freezer of Vegetable leaves processing line

Raw Okra

Raw Green Beans

Raw Peas

Raw Spinach

Raw Molohkia

Blanched Okra

Blanched Green Beans

Blanched Peas

Blanched Spinach

Blanched Molohkia

Frozen Okra

Frozen Green Beans

Frozen Peas

Frozen Spinach

Frozen Molohkia