STUDIES ON IMPACT OF PROCESSING ON PHYSIOCHEMICAL AND BIOCHEMICAL PROPERTIES OF FRUITS AND VEGETABLES

THESIS SUBMITTED BY

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List of Publication/ Seminar/ Conferences and Workshops

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PROFORMA – 1

"Statement of Originality"

I **Arjuma Sultana** registered on **02/03/2016** do hereby declare that this thesis entitled **"Studies on impact of processing on physiochemical and biochemical properties of fruits and vegetables"** contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I havefully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 9%.

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PROFORMA – 2

CERTIFICATE FROM THE SUPERVISORS

This is to certify that the thesis entitled " Studies on impact of processing on physiochemical and biochemical properties of fruits and vegetables " submitted by Arjuma Sultana, who got her name registered on 02/03/2016 for the award of Ph. D. (Engg) degree of Jadavpur University is absolutely based upon her own work under the supervision of Prof. Uma Ghosh, Professor of Department of Food Technology & Biochemical Engineering. Jadavpur University, West Bengal and neither her thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

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DECLARATION

I hereby declare that this thesis entitled "**Studies on impact of processing on physiochemical and biochemical properties of fruits and vegetables**" is the bonafide report of original research work carried out by me under the guidance of Prof. Uma Ghosh, Department of Food Technology and Biochemical Engineering, Jadavpur University and no part thereof has been included in any other thesis submitted previously for the award of any degree.

Place: Jadavpur University, Kolkata .....

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ARJUMA SULTANA

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Objective of the Study

India is blessed with abundant of fruits and vegetable crops as these fruits and vegetables are seasonal and perishable so we have to preserve those scientifically to reduce their wastage. Hence, we apply the preservation technique dealing with the process of treating and handling fruits to stop the loss of quality, edibility and their nutritive value. Maintenance of nutritive value, texture and flavor are important in preserving its values as food. Their nutritional value is highest when they are fresh, but it is not always possible to consume them immediately. During the harvest season, their plentily available but at other times it is scarce. Moreover, most fruits and vegetables are only edible for a very short time, unless they are promptly and properly preserved

Citrus fruits characteristically high in citric acid include oranges, grapefruits, lemon etc. Citrus fruits contain a range of key nutrients including high levels of vitamin C and significant amounts of dietary fibre, beta-carotene and folic acid. They have a low ratio of sodium to potassium and are low in fat and dietary energy, making them nutrient dense, energy-dilute foods with a low glycaemic index. Citrus fruits are also extremely rich in antioxidants. In recent years increasing attention has been given to the sum of biologically active elements found in citrus fruits particularly their plant-based non-nutrients called phytochemicals – because of the role they might play in preventing a range of chronic disease conditions including cancer and heart disease. Citrus fruits contain hundreds of phytochemicals and there is increasing interest in the possibility that these substances contribute to optimal health and may protect against some of the common chronic diseases such as cancer and cardiovascular disease, degenerative eye and cognitive conditions, and general damage caused by ageing.

Modified atmosphere packaging (MAP) of fresh fruits and vegetables is based on modifying the levels of O_2 and CO_2 without addition of any preservative in the atmosphere produced inside a package sealed with some type of polymer film. The growth of organisms is thereby reduced and the life of the product is thus extended. Additionally, the desired atmosphere can reduce the respiration rate, and ethylene production, physiological changes.

Dehydration of fruits and vegetables is one of the oldest and easiest methods of food preservation. Drying food is also a way of preserving seasonal foods for later use. The basic objective in drying food products is the removal of water in the solids up to a certain level, at which microbial spoilage is minimized. The wide variety of dehydrated foods (dried fruits, dry mixes and soups, etc.), which today are available to the consumer and the interesting concern for meeting quality specifications; emphasize the need for a thorough understanding of the operation. Dehydrated products can be used in many processed or ready-to-eat foods in place of fresh foods due to several advantages such as convenience in transportation, storage, preparation and use. Dehydrated products need to be rehydrated before consumption or further processing.

Rehydration i.e., moisturizing of dry material is influenced by several factors, grouped as intrinsic factors and extrinsic factors. Physical and chemical changes that take place during drying affect the quality of the dehydrated product. Rehydration can be considered as a measure of the injuries to the material caused by drying and treatments preceding dehydration.

Osmotic dehydration has received greater attention in recent years as an effective method for preservation of fruits and vegetables. It facilitates processing of fruits and vegetables with retention of initial fruit characteristics as color, aroma, texture and nutritional composition. It is less energy intensive than air or vacuum drying process because it can be conducted at low or ambient temperature. They are the potential source of dietary antioxidants which play a crucial role in preservation of various diseases. Polyphenols possess anticarcinogenic, antiimplammatory. antihepatotoxic, antibacterial, antiviral, antiallergic, antithrombic and antioxidative effect. Fruits and vegetables are rich in polyphenols. However processing is believed to be responsible for losses in natural antioxidants activities. Thus, nutritional quality and health promoting capacity of fruits and vegetables due to processing, spoilage have become increasingly a serious problem.

Diets rich in vegetables and fruit have been linked with lower rates of cancer and coronary heart disease. Plant-based phenols, flavonoids, isoflavones, terpenes, glucosinolates, and other compounds that are present in the everyday diet are reported to have antioxidant and anticarcinogenic properties and a wide spectrum of tumor-blocking activities. The search for the mechanisms of chemoprotection has focused on the biological activity of compounds found in cruciferous and green leafy vegetables, soybeans, citrus fruit, green tea, and red wine. These

compounds, known as phytochemicals or phytonutrients, hold major promise in the creation of designer foods for the dietary prevention of chronic disease.

However, bitterness is usually unpleasant, but sometimes desirable in moderate amounts, and is perceived predominantly at the back of and sometimes along the sides of the tongue. Bitterness of citrus juices significant quality and acceptability of juice and juice based product. So, removal of bitterness is important to improve taste, quality as well as acceptability of the product. Therefore, it is of great importance to study the impact of processing and storage on quality of food.

Plan of Work

- Dehydration kinetics of red Amaranth leaves (*Amaranthus tricolor L.)* using thin-layer drying technology.
- Rehydration kinetics of thin layer -dried red Amaranth (*Amaranthus tricolor L.*) leaves.
- Osmodehydration of pineapple cuboid and its storage stability
- Modified atmospheric packaging of red Amaranth (*Amaranthus tricolor L.*) leaves.
- Extraction and Identification of natural color of red amaranth leaves by different solvent
- Application of natural color extracted from red amaranth leaves

Introduction

Due to India's varied environment, various kinds of fresh fruits and vegetables are always available. After China, it produces the most fruits and vegetables worldwide. According to the National Horticulture Database's (Second Advance Estimates) report, India produced 200.45 million metric tonnes of vegetables and 102.48 million metric tonnes of fruits in 2020–21. Fruits were grown on 9.6 million hectares of land while vegetables were grown on 10.86 million hectares. The largest fruit-producing states in the nation are Andhra Pradesh, Maharashtra, Uttar Pradesh, Madhya Pradesh, Gujarat, Karnataka, and Tamil Nadu, whereas the major vegetable-producing states are Uttar Pradesh, West Bengal, Madhya Pradesh, Bihar, Gujarat, Maharashtra, and Odisha (according to the Third Advance Estimates for 2020–21, in order of production).

Essential nutritional components like vitamins, minerals, and dietary fibre can be found in large quantities in fruits and vegetables. Fresh fruits and vegetables are considered highly perishable commodities since they contain more than 80% moisture [1]. The nutritional importance of FAVs has long been acknowledged. They are not only excellent resources of fibre, essential fatty acids, and minerals but also special sources of vitamins (C, E, B, and folic acid). Specifically,they are an excellent source of bioactive phytochemicals. They are considered as being low in energy and increase appetite since they contain a lot of indigestible fibre. Because of these factors, the basis of the majority of food pyramids recommends eating FAV and plants in particular [2]. FAV are the most crucial element of some people's diets and help them lead healthy lives [3]. In fact, a growing body of research indicates that eating regularly from a diet high in phytochemicals lowers the risk of many chronic diseases in people and lengthens and improves their quality of life [4, 5]. Numerous epidemiological studies have associated the use of FAV and/or its components to favorable health outcomes. For instance, numerous cohorts, case-control, and intervention epidemiological studies demonstrate the positive effects of FAV. Generally, there is a significant correlation between FAV consumption and cardiovascular disease [6, 7], chronic inflammatory diseases [8], diabetes [9, 10], obesity [11], neurodegenerative diseases etc. [12]. But there is less convincing evidence for this effect in cancer [13]. Recently, considerable scepticism has been expressed regarding the relationship between FAV consumption and the prevention of coronary heart disease because it is sometimes confused with other general healthy lifestyle habits like quitting smoking and drinking less alcohol etc.[14].

Red amaranth is a superb vegetable with rosy veined dark green leaves or completely red to purple leaves, reasonable for filling in warm climate, in which youthful leaves and stems can be collected occasionally. Red amaranth is likewise particularly nutritious, plentiful in effectively absorbable minerals i.e., iron and calcium, as well as protein, L-ascorbic acid, and beta-carotene[15] .The nutrients and minerals present in plants as natural or synthetic antioxidants have been connected to eliminating unsafe atoms called free radicals in the body to help protest against contamination and different circumstances, including serious eye disorders, leukemia, heart disease, muscle degeneration [16].White (light green), dark green, crimson, purple, and variegated are just a few of the leaf colours that can be found on a tricolour plant [17] .The primary usage for amaranths is as a leafy vegetable. Leafy vegetables contain polyphenols, which have been shown to protect against a number of chronic diseases, including as diabetes, cardiovascular disease, and cancer [18]. According to preliminary research, Amaranthus leaves are significant sources of antioxidants, but it's critical to identify the principal antioxidants and the antioxidant activity, particularly for red amaranth [19]. The phenolic content and antioxidant properties of red amaranth cultivars with various natural colors may vary. Moreover, the total phenolic content and antioxidant activity of cultivars with the same flesh colour may vary. The antioxidant capacities and phytochemical contents of different dietary products have been linked in several research but red amaranth does not contain this information [20]. To raise consumer knowledge of the abundance of beneficial phytochemicals contained in this nutrient-dense food, data on the total phenolics and antioxidant activity of red amaranth would be valuable.

Pineapple (Ananas comosus) is the most commercially significant tropical fruit crops in the world. It is known as the "queen of fruits" due to its excellent flavour and taste [21]. After citrus and bananas, pineapple is the third most significant tropical fruit in the world [22]. Fresh, cooked, juiced, and preserved pineapples are all consumed or served. This fruit is seasonal and extremely perishable. A good amount of bromelin, a protein-digesting enzyme, citric acid, malic acid, and vitamins A and B are also present in mature fruit, which also includes 14% of sugar [23]. The ingredients in pineapple juice vary based on the region, time of year, harvesting method, and process. The fruit's flavour is refreshing because of the fruit's sugar and acid balance. The pineapple is a beautiful tropical fruit that offers a tonne of health advantages along with exceptional juiciness and bright tropical flavour. The significant amounts of calcium, potassium, vitamin C, carbohydrates, dietary fibre, water, and other minerals found in

pineapple are helpful for the digestive system, aid in maintaining a healthy weight, and promote balanced nutrition.

Leafy vegetables are perishable commodities. The best quality product can be obtained by optimizing the processing and storage conditions. The food must be preserved while maintaining its nutritional content, texture, colour, and flavour. Many preservation techniques are available. Some are thermal and some are non-thermal. Dehydration is one of the earliest and least expensive thermal preservation technique for preserving fruits and vegetables. Osmodehydration, blanching, modified atmospheric packaging etc are the most commonly used non-thermal process. The utilization of dried foods as processed or ready-to-eat food is very popular today. Dehydrated foods are easy to handle to transport, storage and use.

Recent years have seen a lot of interest in the preservation of fruits and vegetables' freshness through the submersion of water-containing cellular components in an osmotic solution. Due to the solute gain and water loss, it causes the creation of intermediate moisture products with lower water activity. Recent years have seen a lot of interest in the preservation of fruits and vegetables' freshness through the submersion of water-containing cellular components in an osmotic solution. Due to the solute gain and water loss, it causes the creation of intermediate moisture products with lower water activity. The technique significantly reduces the chemical, physical, and biological activity that causes foods to degrade, hence extending the storage stability of fresh food. Phase change has been avoided in this process because moisture is removed from the product at room temperature through diffusion. It utilizes less energy than other traditional drying techniques and enhances the nutritional value and sensory qualities of foods.

Osmotic dehydration has subsequently been combined with a number of additional techniques, including pulsed high electric field, high hydrostatic pressure, microwave heating, pasteurization, ultrasound, centrifugal force, vacuum, and gamma irradiation, blanching. Osmotic agent, time and temperature, the solution to sample ratio, the concentration of the solute, agitation, and the shape of the materials, affect osmotic dehydration process. These methods have been used before, during, or after osmotic therapy to improve the efficiency of osmotic dehydration by enhancing mass transfer rate and cell membrane permeability. Consequently, these processes shorten the drying process, cut down on additional energy expenses, and enhance the quality of fruits and vegetables while they are being stored.

Due to its potential for the food processing industry, osmotic dehydration of food has recently received much interest. Osmotic dehydration is typically used to substantially remove water from plant tissues by submerging them in a hypertonic solution. A product normally won't achieve a low-enough moisture content by osmotic dehydration to be deemed shelf-stable. Food dehydration is typically accomplished through an interaction called osmotic dehydration. It prompts alluring items that are prepared to eat or can be applied as a pretreatment to the following system, for example, drying or freezing. Water is a principal constituent of food sources, which influences food security, microbial as well as compound. It is liable for the customer view of quite a large number organoleptic qualities like juiciness, elasticity, tenderness and texture. In food industry, food and food products are preserve by using dehydration process to remove the water from the raw materials. The bringing down of water action can be accomplished in two ways, either by the expansion of humectants or by the expulsion of dissolvable, for example, water. Now a days, researcher focused on the improvement of product quality of preserved food products. Osmotic dehydration is the cheapest and easiest method to obtain better food products by removing water at low temperature [24]. It has been broadly utilized as a pretreatment step in dehydration process since it can diminish generally the energy prerequisite for additional drying process [25]. To fulfill the developing market interest for products in a fresh like state, insignificant handling like osmotic lack of hydration will be progressively utilized. The dynamic exploration in the area of osmotic drying out of organic products is proceeding from one side of the planet to the other.

The recent trends in the market for freshly cut, minimally processed, or "ready to eat" green vegetables has grown massively. Postharvest disorders are caused by the sorting, cutting, and packaging techniques used to make minimally processed leafy vegetables [26]. These conditions has an great impact on both internal and external quality as well as the freshness of the leafy vegetables. The freshness, color, damages and the size of the minced leaves of vegetable crops is crucial for the purpose of marketing. [27,28]. Fresh fruit and vegetable products can be packaged using modified atmosphere (MAP) without the use of preservatives by changing the levels of O2 and CO2. The preferred environment might lessen physiological changes like ethylene production and respiration rate. It is highly advantageous for transportation of agricultural commodities with minimum loss. Presence of considerable amount of vitamin C, β-carotene, anthocyanin and antioxidant makes red amaranth leaves nutritionally rich leafy vegetables [29, 30, 31].

Dehydration prevents the texture, color and flavor losses of leafy vegetables due to low moisture content. Storage is very important to extend the self-life and get the best quality product. Now a day's dried foods are very trendy, and it's used as processed food or ready to-eat foods. Dehydrated products are mainly rehydrated when using the product. Rehydration or the water absorption characteristics is a process of moisturizing the dried product made by an abundant amount of water. Rehydration is influenced by some intrinsic factors such as chemical composition of the product, pre-drying treatment, dehydration process, moisture removal conditions, and method of storage after drying. As well as some extrinsic factors like composition of dipping solution, condition of rehydration and temperature. During drying process, the quality of the dehydrated products depends on the effects of chemical and physical changes in raw material. Dehydration and rehydration process is reversible when the pre-drying and post-drying product quality is almost the same. Immersion of dry material in water results in many changes due to water imbibition's and loss of solutes. The effectiveness of the rehydration process depends on the drying method and the pre-treatment process of the initial material. The rehydration rate depends on the rehydration method (shaking or agitation) and temperature, and quantity of absorbed water. When the temperature increases the rehydration process is faster than the lower temperature due to quick cell wall and tissue disruption. The kinetic study is most important for understanding the rehydration process. It is important to know how water absorption can be accomplished and how it will be influenced by processed variables. Fathima et al.,2001 described the rehydration characteristics of some microwave-dried greens and Mujaffar and Loy 2016 investigated the effect of temperature on the rehydration behavior of microwave-dried Amaranth (Amaranthus dubius) leaves[32,33]. Ergun et al. 2016 studied the characteristics of rehydrated-freeze-dried kiwi slices at different ratios of sample and distilled water [34]. There are studies dealing with the effect of rehydration conditions on the rate of water absorption as well as the loss of soluble solids during rehydration.

Color is thought to be the major sensory element that has played a significant part throughout history in determining whether or not food is accepted in order to improve its real quality and appearance. Plant pigments, the ideal natural source of color, have a great deal of promise to replace many synthetic colouring agents. Plant pigments have a lot to offer the food sector, and those perspectives are positive. There has been an increase in the use of natural colourants. Renowned for their therapeutic and medicinal benefits across the world. Consequences in addition to their high toxicity [35,36]. Anthocyanins are polyphenols with known antioxidant

activity which are responsible for the red, blue, purple colors of plant assets like organic products, blossoms, and leaves. Anthocyanins are mostly found in berries, such blackberries, grapes, and blueberries, as well as some vegetables, like carrot, egg-plants and avocados. Other foods that are important parts of the human diet include oranges, elderberries, olives, red onions, figs, sweet potatoes, mangoes, purple corn, pomegranates, and red amaranth. Anthocyanins may be used in the food, drug, and cosmetic sectors. In the food sector, the vegetable pigments carotenoids and anthocyanins are most frequently used. Commercially produced anthocyanin-based colourants for used in food are created from horticulture crops and processing waste. In the literature, there is a wealth of information on the chemistry of anthocyanins, including conventional methods, organized techniques for classifying anthocyanins, calculating their concentrations, and analysing their colour characteristics. Recent research has demonstrated that anthocyanins exhibit a variety of biological actions. They can be employed as antioxidants, which are thought to have several positive health effects. In addition, it contains anti-inflammatory, antibacterial, and anti-carcinogenic properties. Furthermore, they show different impacts on veins, platelets, diabetes, joint pain, and lipoproteins ready to decrease the gamble of coronary heart sicknesses. In the current work, utilization of anthocyanin extracted from red amaranth leaves in food industry.

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Chapter 1

Dehydration kinetics of red amaranth leaves

1.1. Introduction

Red amaranth is a wonderful vegetable with reddish veined dark green leaves or fully red to purple leaves, suitable for growing in warm weather, in which matured leaves and stems can be harvested periodically. It's also extremely healthy, containing high in protein, vitamin C, beta-carotene, and quickly digested minerals like calcium and iron. Natural or synthetic antioxidants found in plants, such as vitamins and minerals, have been associated to the removal of toxic components have been linked to removing harmful molecules called free radicals in the body to help fight against infection and other conditions, including chronic eye disorders, leukemia, heart disease, muscle degeneration[2]. A tricolor has a variety of leaf colors such as white (light green), dark green, red, purple and variegated [3]. Green and red amaranths are mainly used as leafy vegetables. Leafy vegetables are sources of polyphenols, which prevents many chronic diseases, including cancer, cardiovascular diseases, and diabetes, has been well documented [4].

Initial researchers discovered that amaranthus leaves are the significant sources of antioxidant but it is crucial to identify the main antioxidants and their antioxidant activity, particularly for red amaranth [5]. Red amaranth cultivars of different flesh color may differ in their phenolic content and antioxidant properties. In addition, cultivars with the same flesh color may differ in total phenolic content and antioxidant activity. Several studies have reported correlations among the antioxidant activities and phytochemical concentrations of various food commodities; however, this type of information is not available for red amaranth. Information on the total phenolics and antioxidant activity of red amaranth would also be helpful for increasing the awareness of consumers regarding the level of beneficial phytochemicals present in this nutritious vegetable [6].

Leafy vegetables are perishable commodities. Hence optimization of parameters for processing and storage is very important to get best quality product. Maintenance of nutritive value, texture, color and flavour are important in preserving the food. Dehydration is the most inexpensive and oldest preservation techniques of fruits and vegetables. Now a day’s dried foods are very trendy

and it's used as processed or ready- to- eat foods. Dehydrated foods are easy to handle to transport, storage and use.

The objective of this work was the evaluation and the modeling of the drying kinetics of mass transfer during thin layer drying techniques of red amaranth leaves, and the analysis of the influence of hot-air oven conditions on the kinetic constants of the proposed models. A bunch of mathematical equations that can adequately explain the system and it forms the basis of the modelling principle. In order to calculate the process parameters as a function of time at any point in the dryer depending on the primary condition, these equations must be performed [7]. Consequently, a simulation model is a crucial tool for predicting the effectiveness of drying systems.

1.2 Materials and methods

1.2.1 Preparation of sample for drying

1.2.1.1 Control sample

Undamaged matured fresh leaves of red amaranth were taken, and then properly clean it in tap water for removing foreign matters like mud, dirt, chaff and immature leaves. The surface moisture was removed by using muslin cloth and after that it air dried at room temperature. Then the leaves were dried at three different temperature 50^0C, 60^0C and 80^0C in a tray drier for 4hour.

1.2.1.2 Chlorinated sample

Undamaged matured fresh leaves of red amaranth were taken and properly cleaned it in tap water for removing foreign matters like mud, dirt, chaff and immature leaves. The surface moisture was removed by using muslin cloth and then it air dried at room temperature. After that these leaves were dipped in chlorinated water (5 drop of Zeoline-200 in 1L distilled water) for 5min. Then, the leaves were drained out and the surface water on the wet red amaranth leaves and removed with a muslin cloth. The red amaranth leaves were dried at three different temperature 50^0C, 60^0C and 80^0C in a tray drier for 4 hours.

1.2.1.3 Water blanched sample

Undamaged matured fresh leaves of red amaranth were prepared as mentioned above. These leaves were blanched at 80^0 C temperatures for 3min. After that the blanched leaves were placed under running tap water immediately to cool in ambient temperature and the surface water on the wet leaves of red amaranth was removed with a muslin cloth. The red amaranth leaves were dried at three different temperatures 50^0C, 60^0C and 80^0C in a tray drier for 4hours.

The samples obtained after drying at different temperatures were subjected for further studies.

1.2.2 Analytical parameters

Moisture Content was calculated according to the method described by Ranganna(1986) and expressed as dry basis[8].

Total phenol content was calculated by Folin-ciocaltue method at a wavelength of 765nm using gallic acid standard described by Singleton and Rossi (1965) and expressed as mg of Gallic acid/mg of red amaranth leaves in dry basis [9].

Antioxidant capacity of red amaranth leaves was calculated by FRAP (ferric reducing/ antioxidant power) assay at a wavelength of 593nm using a spectrophotometer by Benzie and strain as modified by pulido, (Benzie and strain, 1996) and expressed as mg of ascorbic acid/ 100gm of dry red amaranth leaves[10]. The ASAE standard air-oven method, which Henderson and Perry (1976) established, is used to determine the moisture ratio (MR) value in a dry basis [11].

1.2.3 Drying procedure

A laboratory scale tray dryer was used to conduct this drying experiment. The experiments were conducted in three replications at 50^0C, 60^0C and 80^0C of drying air temperature. Before the experiment, the dryer was allowed to work for 15min to reach steady state at desired temperature. Red amaranth leaves were spreaded uniformly on a tray. The mass loss of the sample was measured by using a digital balance with the accuracy of 0.01g, at15min intervals.

1.2.4 Mathematical modeling

During thin layer drying experiment, the moisture ratio of the red amaranth leaves was determined as follows: -

$$MR = \frac{Mt-Me}{M0-Me}$$ ..... Eq. (A.1)

Where, M_0 is the initial moisture content, M_e is the equilibrium moisture content and M_t is the moisture content at time t. All moisture contents are in % of dry basis. From Eq. (A.1) using [12] models we can write the expression of moisture ratio as follows: -

$$MR = \frac{M_t}{M_0}$$ ..... Eq. (A.2)

The different thin layer models and the mathematical expression for these models expressed in Table 1 which used to select the best fitted models for describe the drying curve of red amaranth leaves. Three criteria parameters, R^2 (Coefficient of determination) and RMSE (Root mean square error) and SSE (sum of squares due to error) were used to determine the adequacy of the fit.

Table 1.1. Six different thin layer drying models applied to describe the drying curve of red amaranth leaves

Model Name	Model Equation	Reference
Newton	MR = exp (–kt)	[13]
Page	MR = exp ($–kt^n$)	[14]
Modified page	MR =exp[$(-kt)^n$]	[15]
Henderson and pabis	MR = a exp (–kt)	[16]
Logarithmic	MR = a exp (–kt) + b	[17]
Midilli	MR = a exp ($–kt^n$) + bt	[18]

1.2.5 Moisture diffusivity

Diffusivity is illustrated with the only physical mechanism to move the water to the surface during the drying process is Fick's diffusion equation [19, 20]. Effective moisture diffusivity depends on composition, moisture content, temperature and porosity of the material. It was utilized as a result of the lack of knowledge regarding the mechanism of moisture transport during drying and the complexity of the procedure [21]. The coefficient of effective diffusion

was determined by using the analytical solution of the equation of the second law of fick for flat slab. The effective moisture diffusivity was calculated by using Crank (1975) equation is as following: -

$$MR = \frac{Mt-Me}{M_0-M_e} = \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[-(2n+1)^2\pi^2\ \frac{D_{eff}}{L^2}t\right] \text{.....Eq.(A.3)}$$

Where, D_{eff} is the effective moisture diffusivity and expressed as m^2/ s, L is the full thickness of red amaranth leaves and t is the drying time (min), n is a positive integer [22]. For long drying time, the Eq. (A.3) can be simplified as Eq. (A.4) by taking the first term of series solution and expressed in alogarithmic form;

$$MR = \frac{8}{\pi^2}\exp\left[-\ \frac{\pi^2\ .\ D_{eff}}{4L^2}\ t\right] \text{ ..... Eq.(A.4)}$$

Generally, The moisture diffusivities are calculated by plotting ln(MR) versus drying time to calculate effective diffusivity, a plotted non- linear relationship between the drying time and ln (MR) gives a straight line with a slope of:

$$\ln MR = \ln\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2\ .\ D_{eff}}{4L^2}\ t\right) \text{ ..... Eq. (A.5)}$$

$$\text{Slope} = \pi^2\frac{D_{eff}}{4l^2} \text{ ..... Eq. (A.6)}$$

The empirical values of effective moisture diffusivity (D_{eff}) for different temperature for different pretreated red amaranth samples showed in Table (1.3.2).

1.2.6 Activation energy

In diffusivity model the activation energy was calculated by the Arrhenius equation (1889)

$$D_{eff} = D_0\exp\left(-\frac{E_a}{R.T}\right) \text{ .....Eq. (A.7)}$$

Where, D_0 is Arrhenius constant (m^2/s), R is the universal gas constant (8.314*10^-3 kJ/mol. K), Ea is the activation energy and T_{abs} is the absolute temperature (K). The value of activation energy was expressed as the graphical representation of ln (D_{eff}) against $1/T_{abs}$. The activation

energy was calculated using the Arrhenius equation Eq. (A.6). Logarithm of D_{eff} as a function of the reciprocal of absolute temperature (T) was plotted.

1.3 Results and Discussion:

1.3.1 Drying curve

Moisture ratio Vs time curves for thin-layer drying techniques at 50^0 C, 60^0 C and 80^0 C temperatures are shown in Figure 1.1, Figure 1.2 and Figure 1.3, respectively. At 50^0 C curves showed the decreasing trend of drying process. After completion of drying maximum moisture ratio was observed with chlorinated sample more non-blanching sample than water blanching sample. Similar result was observed at 60^0 C. At 80^0 C moisture ratios was observed with chlorinated, control more water blanching sample.

Table 1.2.1: Moisture ratio verses time for 50˚C temperature dried normal, chlorinated and processed sample

Time	Normal Sample	Chlorinated sample	Processed sample
0	1.00	1.00	1.00
15	0.851	0.886	0.896
30	0.710	0.751	0.756
45	0.590	0.571	0.565
60	0.489	0.417	0.416
75	0.431	0.339	0.307
90	0.370	0.267	0.230
105	0.316	0.205	0.173
120	0.261	0.176	0.143
150	0.194	0.153	0.120
180	0.159	0.129	0.104
210	0.143	0.106	0.082
240	0.120	0.083	0.066
270	0.094	0.072	0.046
300	0.083	0.067	0.040

330	0.075	0.061	0.040
360	0.075	0.061	0.040
390	0.075	0.061	0.040

Table 1.2.2: Moisture ratio verses time for 60˚C temperature dried normal, chlorinated and processed sample

Time	Moisture Ratio	Chlorinated Sample	Processed Sample
0	1.00	1.00	1.00
15	0.769	0.846	0.898
30	0.548	0.697	0.780
45	0.311	0.575	0.681
60	0.170	0.446	0.547
75	0.106	0.302	0.412
90	0.084	0.202	0.280
105	0.071	0.166	0.201
120	0.064	0.113	0.151
135	0.049	0.095	0.114
150	0.049	0.071	0.101
180	0.049	0.049	0.088
210	0.049	0.039	0.071
240	0.049	0.027	0.057
270	0.049	0.027	0.054
300	0.049	0.027	0.054

Table 1.2.3: Moisture ratio verses time for 80˚C temperature dried normal, chlorinated and processed sample

Time	Normal Sample	Chlorinated Sample	Processed Sample
0	1.00	1.00	1.00
15	0.803	0.758	0.811
30	0.516	0.518	0.580
45	0.298	0.339	0.388
60	0.164	0.219	0.270
75	0.065	0.144	0.172
90	0.036	0.098	0.112
105	0.036	0.0768	0.081
120	0.036	0.061	0.058
135	0.036	0.049	0.058
150	0.036	0.049	0.058
180	0.036	0.049	0.058
210	0.036	0.049	0.058
240	0.036	0.049	0.058
270	0.036	0.049	0.058
300	0.036	0.049	0.058

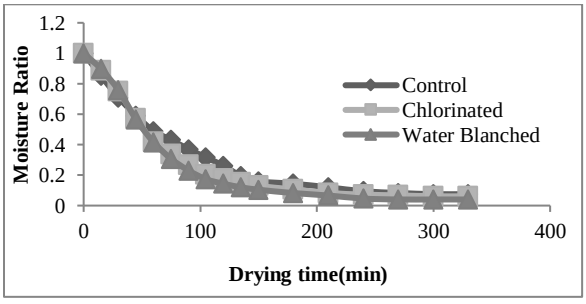

Figure 1.1: Drying kinetics of red amaranth leaves at 50^0 C

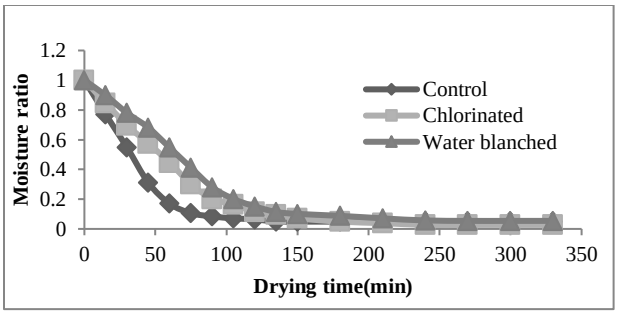

Figure 1.2: Drying kinetics of red amaranth leaves at 60^0 C

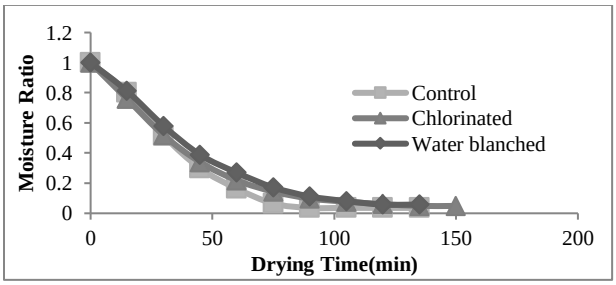

Figure 1.3: Drying kinetic of red amaranth leaves at 80^0 C

The effect of thermal treatment on total phenol content of Control, chlorinated and water blanched red amaranth leaves sample at 50^0 C, 60^0 C and 80^0 C temperature were shown in Figure 1.4, Figure 1.5 and Figure 1.6 respectively. At 50^0 C temperature control red amaranth leaves sample was the higher total phenol content than chlorinated and water blanching sample after completion of drying. Similar result was observed at 60^0 C and 80^0 C.

Table 1.2.4: Effect of dehydration of red amaranth leaves at 50° C on total phenol content

Time	**Normal**	**Clorinated**	**Processed**
0	0.0305	0.0334	0.0403
1	0.0298	0.0315	0.033
2	0.0286	0.0296	0.0338
3	0.0262	0.0272	0.0309
4	0.0249	0.0261	0.0289

Table 1.2.5: Effect of dehydration of red amaranth leaves at 60° C on total phenol content

Time	Normal	Clorinated	Processed
0	0.0292	0.0326	0.0392
1	0.0276	0.0282	0.0354
2	0.0241	0.0274	0.0332
3	0.023	0.0266	0.0299
4	0.0224	0.0234	0.028

Table 1.2.6: Effect of dehydration of red amaranth leaves at 80° C on total phenol content

Time	Normal	Clorinated	Processed
0	0.0284	0.0311	0.0384
1	0.0237	0.0263	0.0301
2	0.0232	0.0245	0.0292
3	0.0226	0.0236	0.0276
4	0.022	0.0228	0.0255
5	0.012	0.0212	0.0245

Table 1.2.7: Effect of dehydration of red amaranth leaves at 50° C on antioxidant activity

Time	Normal	Clorinated	Processed
0	0.461	0.27	0.286
1	0.422	0.252	0.265
2	0.33	0.217	0.222
3	0.283	0.174	0.187
4	0.274	0.126	0.156
5	0.235		

Table 1.2.8: Effect of dehydration of red amaranth leaves at 50° C on antioxidant activity

Time	Normal	Clorinated	Processed
0	0.435	0.257	0.261
1	0.383	0.217	0.243
2	0.296	0.165	0.183
3	0.252	0.131	0.148
4	0.204	0.1	0.108
5	0.143	0.07	0.1

Table 1.2.9: Effect of dehydration of red amaranth leaves at 50° C on antioxidant activity

Time	Normal	Clorinated	Processed
0	0.443	0.265	0.274
1	0.361	0.186	0.2
2	0.3	0.131	0.13
3	0.221	0.096	0.104
4	0.191	0.0826	0.081
5	0.121	0.0789	0.069

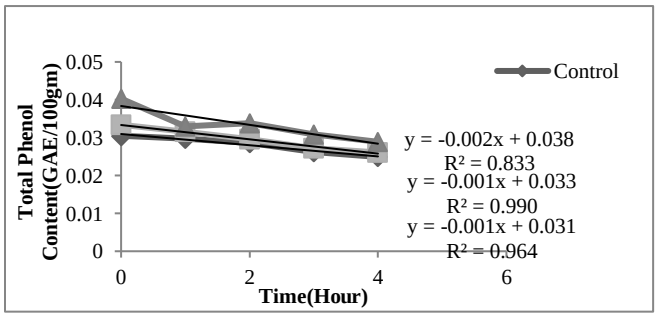

Figure 1.4. Effect of dehydration of red amaranth leaves at 50^0 C on total phenol content

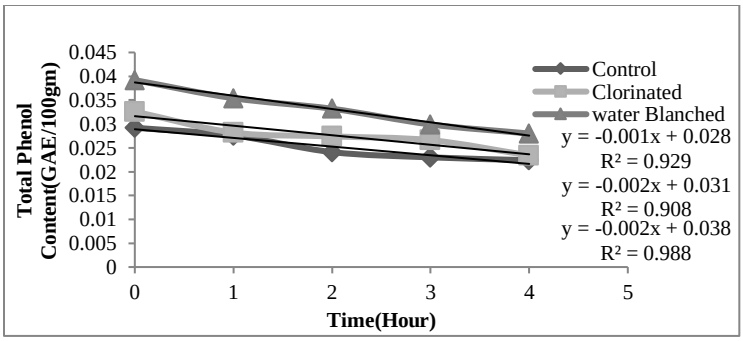

Figure 1.5. Effect of dehydration of red amaranth leaves at 60^0 C on total phenol content

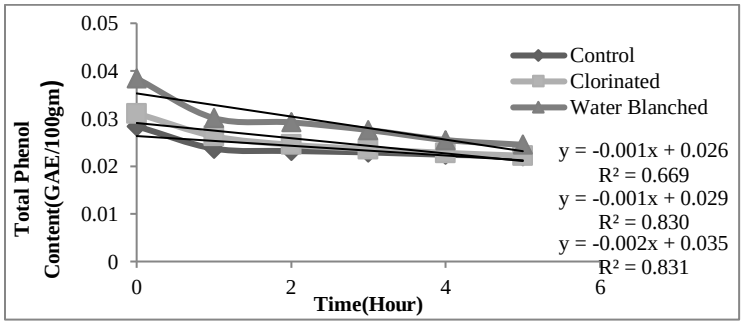

Figure 1.6. Effect of dehydration of red amaranth leaves at 80^0 C on total phenol content

The effect of thermal treatment on antioxidant capacity of control, chlorinated and water blanched red amaranth leaves sample at 50^0 C, 60^0 C and 80^0 C were shown in Figure 1.7, Figure 1. 8 and Figure 1.9. At 50^0 C, control red amaranth leaves sample was the highest antioxidant capacity than chlorinated and then the water blanched sample after completion of drying. Similar result was observed at 60^0 C and 80^0 C.

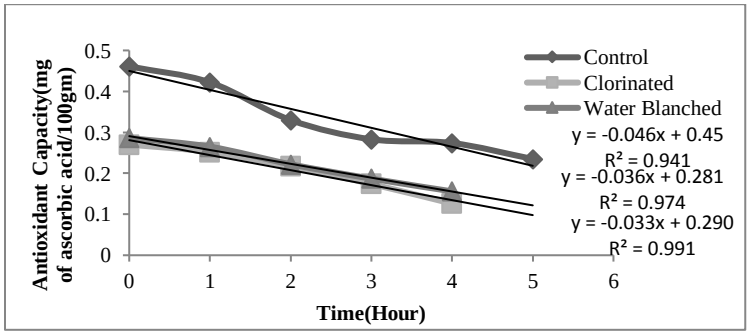

Figure 1.7: Effect of dehydration of red amaranth leaves at 50^0 C on antioxidant activity

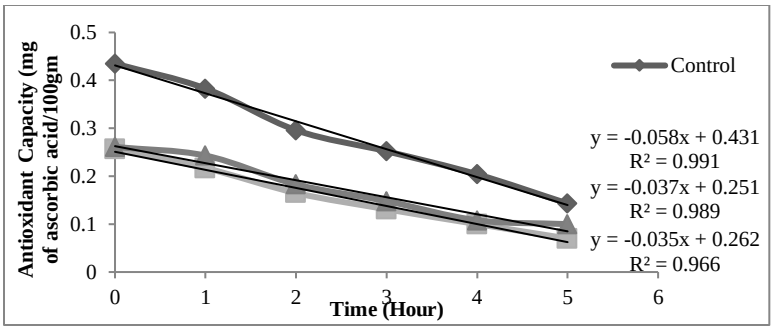

Figure 1.8: Effect of dehydration of red amaranth leaves at 60^0 C on antioxidant activity

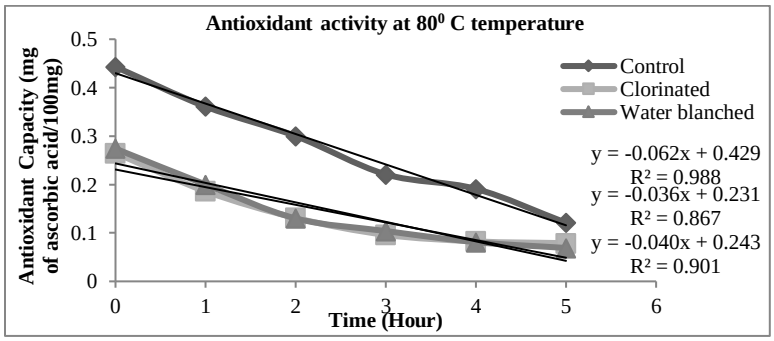

Figure 1.9: Effect of dehydration of red amaranth leaves at 80^0 C on antioxidant activity

1.3.2 Mathematical modeling for red amaranth leaves

The drying process was completed without further change in weight of the sample. Then moisture content began to decrease from 89.39% to 15%. Later, the moisture content data were converted to moisture ratio and fitted to the six thin layer drying models. The results of statistical analysis for mathematical models of pre-treated red amaranth leaves were shown in Table 1.3.1. The best fitted model depended on the highest R^2 value, lowest χ^2 value and RMSE values. On the basis of the empirical result for all the temperature in this process of different pre-treated samples, the Midilli's model is the best fitted model.

Table 1.3.1. Results for six empirical thin layer drying models of red amaranth leaves

Model Names	**Temperature (^{0} C)**	**Sample Type**	**Model Coefficients and Constants**	**SSE**	**RMSE**
	50	Control	K=0.0108	0.011	0.026
		Chlorinated	K=0.0133	0.024	0.039
Newton		Water blanched	K=0.0143	0.031	0.044
	60	Control	K=0.0245	0.018	0.042
		Chlorinated	K=0.0152	0.022	0.041
		Water blanched	K=0.0124	0.052	0.061
	80	Control	K=0.0262	0.0319	0.059
		Chlorinated	K=0.0235	0.0060	0.024
		Water blanched	K=0.0211	0.0131	0.195
	50	Control	K=0.0186, n=0.8797	0.0053	0.02
Page		Chlorinated	K=0.0088, n=1.0937	0.0221	0.039
		Water blanched	K=0.0048, n=1.250	0.016	0.033
	60	Control	K=0.0091, n=1.259	0.0090	0.033
		Chlorinated	K=0.0046, n=1.2753	0.0045	0.02
		Water blanched	K=0.0019, n=1.425	0.0142	0.033
	80	Control	K=0.0041, n=1.487	0.002	0.02
		Chlorinated	K=0.0143, n=1.127	0.002	0.017
		Water blanched	K=0.0079, n=1.244	0.0017	0.014
	50	Control	K=0.0108, n=0.879	0.0053	0.02
		Chlorinated	K=0.0133, n=1.0937	0.022	0.039
		Water blanched	K=0.0141, n=1.251	0.016	0.033
Modified page	60	Control	K=0.0239, n=1.2586	0.009	0.033
		Chlorinated	K=0.0148, n=1.2753	0.004	0.02
		Water blanched	K=0.0123, n=1.4254	0.014	0.033
	80	Control	K=0.0250, n=1.487	0.0028	0.02
		Chlorinated	K=0.0231, n=1.1267	0.0029	0.017
		Water blanched	K=0.0205, n=1.2441	0.0017	0.014

	50	Control	K=0.0104, a=0.976	0.0109	0.028
		Chlorinated	K=0.0140, a=1.044	0.0212	0.037
Henderson		Water blanched	K=0.0153, a=1.068	0.0231	0.039
and					
Pabis	60	Control	K=0.0255, a=1.043	0.0166	0.042
		Chlorinated	K=0.0160, a=1.055	0.0174	0.037
		Water blanched	K=0.0135, a=1.083	0.0392	0.055
	80	Control	K=0.0277, a=1.0652	0.0264	0.057
		Chlorinated	K=0.0241, a=1.0276	0.0050	0.022
		Water blanched	K=0.0220, a=1.0478	0.0099	0.035
	50	Control	K=0.012,a=0.938,b=0.0668	0.0012	0.009
		Chlorinated	K=0.015,a=1.019,b=0.0411	0.0147	0.032
		Water blanched	K=0.016,a=1.058,b=0.015	0.0220	0.039
Logarithmic	60	Control	K=0.025,a=1.046,b=-0.0039	0.0164	0.045
		Chlorinated	K=0.0146,a=1.082,b=0.037	0.014	0.037
		Water blanched	K=0.0126,a=1.1047,b=-0.028	0.0376	0.056
	80	Control	K=0.0243,a=1.106,b=-0.053	0.021	0.056
		Chlorinated	K=0.024,a=1.028,b=-0.0012	0.005	0.024
		Water blanched	K=0.0194,a=1.09,b=-0.055	0.007	0.032
	50	Control	K=0.020,a=1.065,b=0.0001	0.0007	0.008
		Chlorinated	K=0.012,a=1.121,b=0.0002,n=1.067	0.0033	0.017
		Water blanched	K=0.008,a=1.1003,b=0.0002,n=1.182	0.0037	0.017
	60	Control	K=0.0024,a=0.927,b=0.0004,n=1.619	0.0014	0.015
Midilli		Chlorinated	K=0.0015,a=0.927,b=0.00016,n=1.522	0.0019	0.014
		Water blanched	K=0.00038,a=0.9355,b=0.00027,n=1.79	0.0026	0.016
	80	Control	K=0.0028,a=0.99,b=0.00024,n=1.59	0.0002	0.009
		Chlorinated	K=0.011,a=1.0206,b=0.0002,n=1.207	0.000042	0.003
		Water blanched	K=0.0071,a=1.023,b=0.00026,n=1.29	0.00017	0.006

1.3.3 Moisture diffusivity

The moisture diffusivity of red amaranth leaves at three different temperatures for control, chlorinated and water blanched sample was shown in Table 1.3.2. The graphical representation of moisture diffusivity at various temperatures for control, chlorinated and water blanched red amaranth sample was shown in Figures 1.10, Figure 1. 11 and Figure 1.12.

Table 1.3.2. Moisture diffusivity of red amaranth leaves at different drying temperature

Drying Temperature	**Sample**	**Moisture Diffusivity(m^2/s)**
50^0C	Control	-3.2456*10^-7
	Chlorinated	-3.65*10^-7
	Water blanched	-4.057*10^-7
60^0C	Control	-8.925*10^-7
	Chlorinated	-6.491*10^-7
	Water blanched	-4.868*10^-7
80^0C	Control	-11.77*10^-7
	Chlorinated	-8.519*10^-7
	Water blanched	-9.331*10^-7

In our study, three different temperatures of 50^0 C, 60 ^{0} C and 80 ^{0} C were used for tray drying of red amaranth leaves. Control, chlorinated and water blanched samples were used for this drying process. Increased in drying temperature increased the moisture diffusivity of all samples (Table 1.3.2).The moisture diffusivity of control sample was 3.2456*10^-7, 8.925*10^-7 and 11.77*10^-7 at 50 ^{0} C, 60 ^{0} C and 80 ^{0} C respectively. The moisture diffusivity of chlorinated sample was 3.65*10^-7, 6.491*10^-7 and 8.519*10^-7 at 50 ^{0} C, 60 ^{0} C and 80 ^{0} C respectively. The moisture diffusivity of processed sample was 4.057*10^-7, 4.868*10^-7 and 9.331*10^-7 at 50 ^{0} C, 60 ^{0} C and 80 ^{0} C respectively. Similar result was reported by Kadam *et al.*,2011 for thin layer convective drying of mint leaves [23]. The moisture diffusivity (D_{eff}) of mint leaves varied from 1.2325×10^{-10} to $2.6568 \times 10^{-10} m^2/s$ for temperature ranges from 45 to 65^0C. Darvishi *et al.,* (2016) reported the moisture diffusivity of dill leaves as $3.92x10^{-7}$, $4.19x\ 10^{-7}$, $6.22x10^{-7}$ and $7.42x10^{-7}$ for temperature range from 40, 50, 60 and 70 ^{0} C [24]. These values are consistent with

the present estimated D_{eff} values for red amaranth leaves. The graphical representation of moisture diffusivity of red amaranth leaves at three different temperatures and three pretreated samples control, chlorinated and water blanched are shown in Figures 1.10,Figure 1.11 and Figure 1.12.

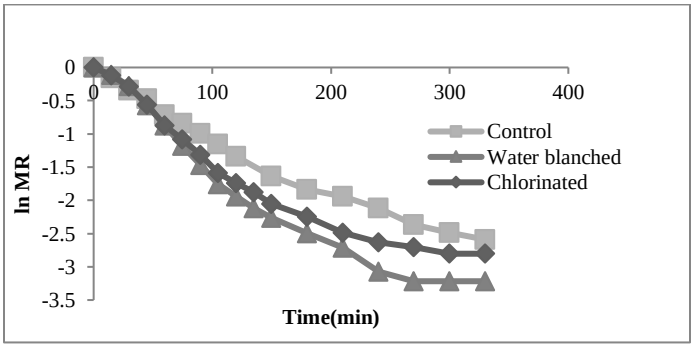

Figure 1.10: Moisture diffusivity of red amaranth leaves at 50^0 C

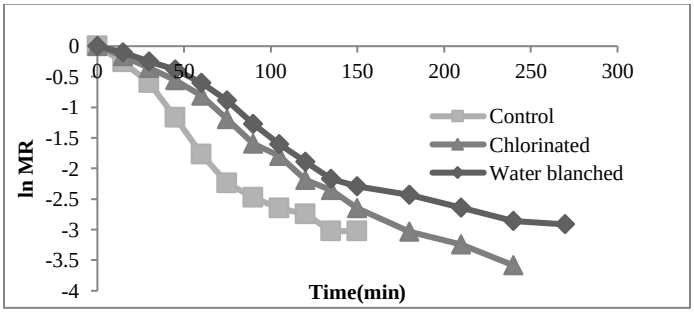

Figure 1.11: Moisture diffusivity of red amaranth leaves at 60^0 C

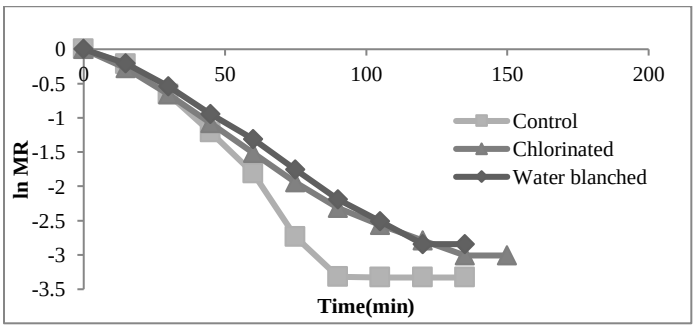

Figure 1.12: Moisture diffusivity of red amaranth leaves at 80^0C

1.3.4Activation energy

The activation energy was expressed as the graphical representation of ln (D_{eff}) against $1/T_{abs}$. The activation energy for diffusivity model was determined by the Arrhenius equation (1889):-

$D_{eff} = D_0 \exp(-Ea/RT)$

And slope=$\pi^2 \frac{D_{eff}}{4l^2}$

The activation energy, Ea, for the Control sample was calculated using the Arrhenius equation's slope to be 11.94 KJ/mol, 12.09KJ/mol for chlorinated sample and 11.43KJ/mol for water blanched sample. Similar result was reported by Kakade and Hathan.,2014 for thin layer convective dehydration kinetics of beet root leaves [25]. The activation energy of blanched and unblanched samples was reported as 15.7913 and 20.9221kJ/mol, respectively for temperature range from 50, 60, 70 and 80^0 C.

Table1.3.3: Activation energy of red amaranth leaves dried at different temperatures

1/T	lnK(Control)	lnk(Chlorinated)	lnK(Water blanched)
3.09	-4.83	-4.96	-4.61
3.003	-3.82	-4.14	-4.42
2.83	-3.59	-3.86	-3.77

The activation energy of various temperatures for chlorinated, water blanched and control samples were shown in Figure 1.13. This figure shows that the chlorinated sample have higher activation energy than the control and water-blanched samples.

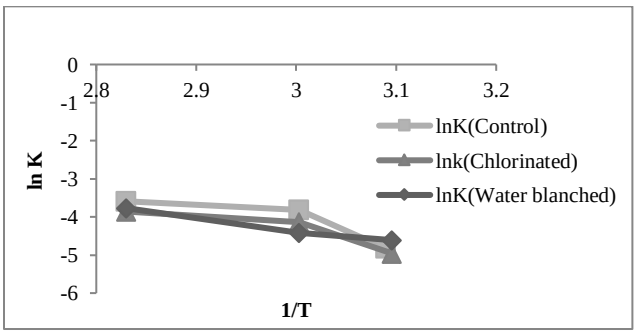

Figure 1.13: Activation energy of red amaranth leaves dried at different temperatures

1.4 Conclusion:

In this study, six thin-layers drying models namely Newton, Page, Modified page, Henderson and pabis, Logarithmic and Midilli models were used to choose an appropriate curvature for the drying curve of red amaranth leaves. The best fitted model to represent the drying kinetics was chosen using the experimental data. The best fitted models depended on the basis of lower sum squared errors (SSE) value and root mean square error (RMSE) and the higher value of correlation coefficient.

Values were obtained in Midilli model. The drying curve of red amaranth leaves was satisfactorily described using the Midilli model. The Midilli *et al.,* (2002) model gave the highest value of R^2 (0.9997). For the control, chlorinated sample and water blanched sample at 50^0C showed the lowest SSE (0.0014) and RMSE (0.015), the lowest SSE (0.0019) and RMSE (0.014) for chlorinated sample and the lowest SSE (0.0026) and RMSE (showed 0.016) for water blanched sample, respectively.

At 80^0C temperature the Midilli's model showed the highest value of R^2 (0.99952), the lowest SSE (0.0002) and RMSE (0.009) for control, the highest value of R^2 (0.99952), the lowest SSE (0.000042) and RMSE (0.014) for chlorinated sample and the highest value of R^2 (0.99952), the lowest SSE (0.00017) and RMSE (0.006) for water blanched sample.

Result showed the activation energy of blanched and unblanched samples for temperature range from 50, 60, 70 and 80 ^{0} C was reported as 15.7913 and 20.9221 kJ/mol respectively. Darvishi *et al.,* (2016) reported the activation energy of dill leaves was16.84 kJ/mol for temperature range from 40, 50, 60 and 70 ^{0} C. During water blanching water can be removed from the sample by less energy due to the cell rupture of skin [24]. The activation energy of chlorinated sample is higher than the control and water blanched sample. These values are consistent with the present estimated D_{eff} values for red amaranth leaves.

The statistical indicator showed that the Midilli's model was the best fitted model to explain the drying kinetics of red amaranth leaves. Among all these models we can consider the Midilli's model is the best fitted model to explain the drying process of different pre-treated red amaranth leaves sample, because in most cases, it represents the lower RMSE value. The similar results

were found in bay leaves (Gunhan *et al.,* 2005) and coriander leaves (Akpinar 2010), Spinach (Doymaz, 2009) and mint leaves (Doymaz, 2006) [26,27,28 &29]. Akpinar (2005) presented similar findings to the ones reported above regarding apples and pumpkin [30]. Venkanna et al., 2019 also represents the similar result for coriander (Coriandrum sativum) leaf [31].

When the drying temperature is raised in thin layer drying process for treated and untreated red amaranth leaves, the drying time is diminished and the drying rate is enhanced. The Midilli's model is the best empirical mathematical model to represents the drying curve of thin layer drying process of treated and untreated red amaranth leaves sample for all the drying temperature. In thin layer drying process blanching applied as a pretreatment to reduce the drying rate of the leaves.

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Chapter 2

Rehydration kinetics of Red amaranth leaves

2.1 Introduction

The nutritional quality of red Amaranth leaves is high, highly proteinaceous, and rich in iron, calcium, vitamin-C and beta-carotene [1]. Leafy vegetables are the sources of polyphenols, which prevent many chronic diseases, including cancer, cardiovascular disease, and diabetes, has been well documented [2]. Amaranth's leaves are an important source of antioxidants [3]. Leafy vegetables are highly perishable. Hence, dehydration techniques are used to prevent nutritional losses. Dehydration prevents the texture, color and flavor losses of leafy vegetables due to low moisture content. Storage is very important to extend the self-life and get the best quality product. Now a day's dried foods are very trendy, and it's used as processed food or ready to- eat foods. Dehydrated products are mainly rehydrated when using the product. Rehydration or the water absorption characteristics is a process of moisturizing the dried product made by an abundant amount of water. Rehydration is influenced by some intrinsic factors such as chemical composition of the product, pre-drying treatment, dehydration process, moisture removal conditions, and method of storage after drying. As well as some extrinsic factors like composition of dipping solution, condition of rehydration and temperature. During drying process, the quality of the dehydrated products depends on the effects of chemical and physical changes in raw material. Dehydration and rehydration process is reversible when the pre-drying and post-drying product quality is almost the same. Immersion of dry material in water results in many changes due to water imbibition's and loss of solutes. The effectiveness of the rehydration process depends on the drying method and the pre-treatment process of the initial material. The rehydration rate depends on the rehydration method (shaking or agitation) and temperature, and quantity of absorbed water. When the temperature increases the rehydration process is faster than the lower temperature due to quick cell wall and tissue disruption. The kinetic study is most important for understanding the rehydration process. It is important to know how water absorption can be accomplished and how it will be influenced by processed variables. Fathima et al.2001 described the rehydration characteristics of some microwave-dried greens and Mujaffar and Loy 2016 investigated the effect of temperature on the rehydration behavior of microwave-dried Amaranth (Amaranthus dubius) leaves [4,5]. Ergun et al. 2016 studied the characteristics of rehydrated-freeze-dried kiwi slices at different ratios of sample and distilled water [6]. There are studies dealing with the effect of rehydration conditions on the rate of water absorption as well as the loss of soluble solids during rehydration.

Rehydration kinetics describes the mechanisms and the influence that certain process variables exert upon moisture transfer [5,6 &7]. A significant proportion of soluble solids may dissolve into the solution during rehydration, which could have an impact on the nutritional value of the food staff and water absorption. The final retention of the dry matter does not influence the temperature of the rehydration medium. In this study we investigate the impact of rehydration temperatures on three pretreated samples dried at four different temperatures. During rehydration the leaching process is described by the mathematical model namely Fick's second law. Now adays, the water uptake and the soluble solids losses during rehydration are described by the Weil bull probabilistic distribution, but during short rehydration, the product quality of the soluble solids is not studied yet. Some researchers reported that the compositions of the soluble materials discharging from vegetables, legumes are determined by using HPLC methods. Leached solids are sometimes contingent from a mass balance for the rehydration of vegetables. The rehydration kinetics of red Amaranth leaves were studied by the water absorbance of the dried leaves at four different temperatures (38°C, 50°C, 60°C and 80°C) and the rehydration curve was described by the moisture content as a function of time.

The mass transfer phenomena are described using the first-order kinetic model assuming

1. Temperature of water is constant

2. Initial moisture content of samples is uniform

$$d(X) / dt = - K_r (X - X_e) \quad \text{……..(Eq 1)}$$

Where,

X = Moisture content of the material during this process (kg/kg Dry basis)

Kr = Rate of rehydration (Per min),

Xe = Equilibrium moisture content of rehydrated material (kg/kg Dry basis)

T= Time period (min).

At saturation condition, the equilibrium moisture content was express as the water concentration. First order kinetics model was the best fitted model to express the experimental data and the result of this kinetic study. In this condition the equilibrium moisture content is not equal to the moisture content of the fresh sample. This indicates that drying process is irreversible. In this experimental condition, rehydration did not reach the higher moisture content, which means during drying structural damage and cell shrinkage occur. As a result, rehydration ability is decreases.

Mathematical model of rehydration kinetics

Rehydration kinetics

$$X = Xe - (Xi - Xe)\, e^{-krt} \quad \text{..... (Eq.2)}$$

Where,

X = Moisture content (kg/kg Dry basis)

Xe = Equilibrium moisture content (kg/kg Dry basis)

Kr = Rate constant of moisture loss (Per min)

Factors affecting the parameters

Rehydration conditions

Water temperature (T, ˚C)

$$K_r = K_0\, (T/60)^n \quad \text{..... (Eq.3)}$$

$$X_e = X_0\, (T/60)^m \quad \text{..... (Eq.4)}$$

Many researchers are covered on this topic and they study on the rehydration kinetics of leafy vegetables like spinach, mint, basil, coriander, and leaves from West American pepper plants, among others. In this work, the rehydration kinetics of red amaranth leaves are described using Peleg's model. Peleg's constants K_1 and K_2 were determine using equations 5 and 6[17]

$M_t = M_0 \pm \frac{t}{K_1 + K_2 t}$ ……. (Eq.5)

$\frac{t}{M_t - M_0} = K_1 + K_2\, t$ ……. (Eq.6)

M_e was calculated from equation 7 using the value of K_2 [8,12,11]

$M_e = M_0 + 1/K_2$ …….. (Eq.7)

2.2Materials and Methods

2.2.1Preparation of sample for drying

2.2.1.2 Normal Sample: Described in Chapter 1

2.2.1.2Chlorinated sample: Described in Chapter 1

2.2.1.3Water blanched sample: Described in Chapter 1

2.2.2Drying procedure: Described in Chapter 1

2.2.3Rehydration Procedure

Samples are dried at four different temperatures through thin layer drying process and the dehydrated samples are rehydrated in a temperature-controlled water bath shaker (manufactured by Sicco Instruments Pvt. Ltd., Kolkata, India) at a constant speed of 60 rpm at 38°C, 50°C, 60°C and 80°C temperature. For each experiment 2g of dehydrated red amaranth leaves were used. Samples were rehydrated in 250 ml Erlenmeyer flasks containing 100ml distilled water. Then the beakers were put into a water bath and withdrawn after 15 min intervals. After specified soaking times, the hydrated leaves were blotted free of excess surface moisture with an absorbent cloth and then air-dried. After that, the weight was measured in a digital weighing machine. Calculate the weight difference to measure the water absorbance by the dehydrated sample. The capacity of dehydrated leaves to absorb moisture is defined as the rehydration ratio.

2.2.4Mathematical Modeling

For this rehydration kinetic study, we are using the linear form of Peleg's equation (Eq. 5) to fit the experimental data within the curvilinear segments of graphs obtained. The rehydration process was done until the difference between two consecutive weights was insignificant. The percentage of moisture content was expressed as the dry basis (% dw). The rehydration ratio was calculated by the ratio between the weight of the rehydrated material and the weight of the dehydrated material, as described by Ranganna, 1986 [14].

2.3 Results and Discussion

Rehydration is widely used as a parameter for dried sample quality. H.feng and J. tang,1998 established that the physical and chemical changes during the dehydration process are influenced by sample composition, pre-treatment process and the processing conditions [15]. The rehydration process shows that the rehydration ratios of dried chlorinated, water blanched, and control samples at 60˚C are higher than those of dried ones at other temperatures. An increase in temperature above 60˚C had an adverse effect on the final rehydration ratio, which decreased with increasing temperature. It indicates the change in the product induced by temperature and perhaps a loss of solids during the rehydration process. Cunningham et al.,2008 reported that 60˚C convection dried potato slice gives the better result[16].

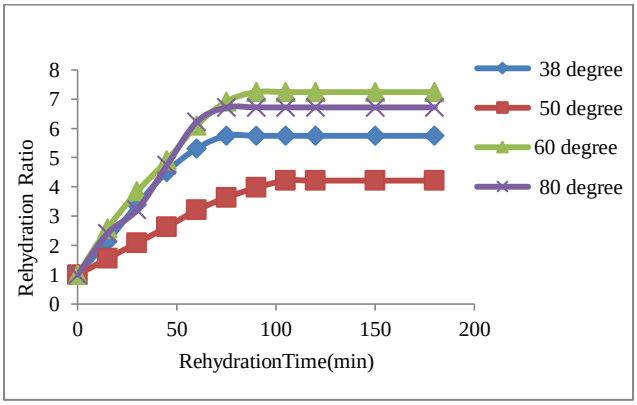

Figure 2.1: Rehydration kinetics of red Amaranth leaves at different temperatures for 50˚C temperature dried Control sample

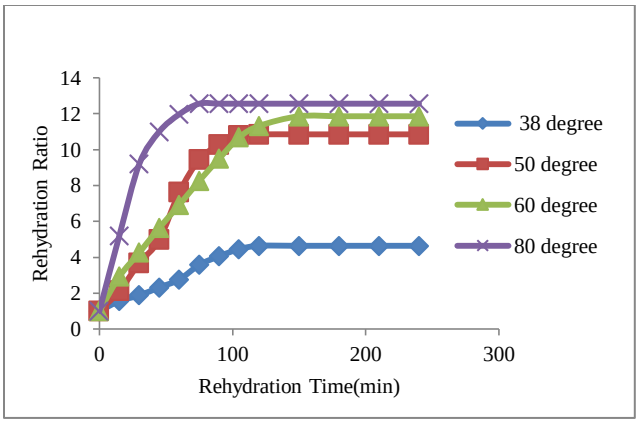

Figure 2.2: Rehydration kinetics of red Amaranth leaves at different temperatures for 50°C temperature dried Chlorinated sample

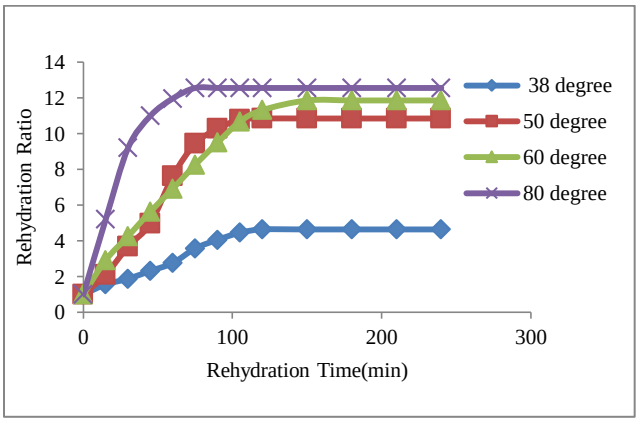

Figure 2.3: Rehydration kinetics of red Amaranth leaves at different temperatures for 50°C temperature dried processed sample

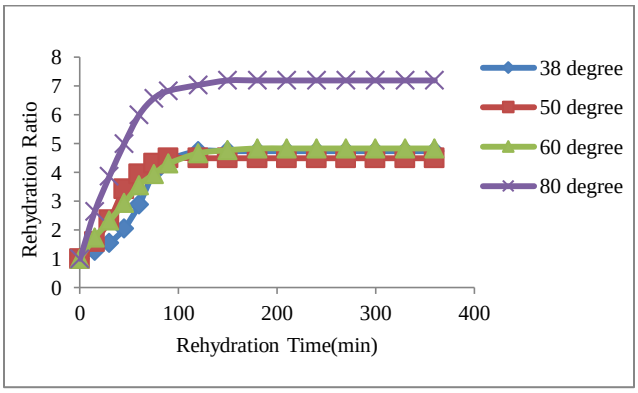

Figure 2.4: Rehydration kinetics of red Amaranth leaves at different temperatures for 60°C temperature dried control sample

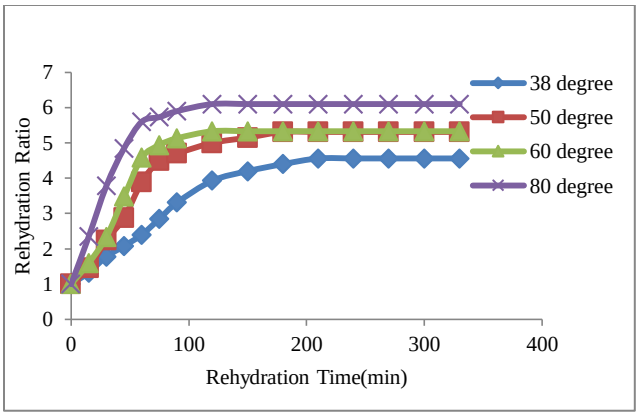

Figure 2.5: Rehydration kinetics of red Amaranth leaves at different temperatures for 60°C temperature dried chlorinated sample

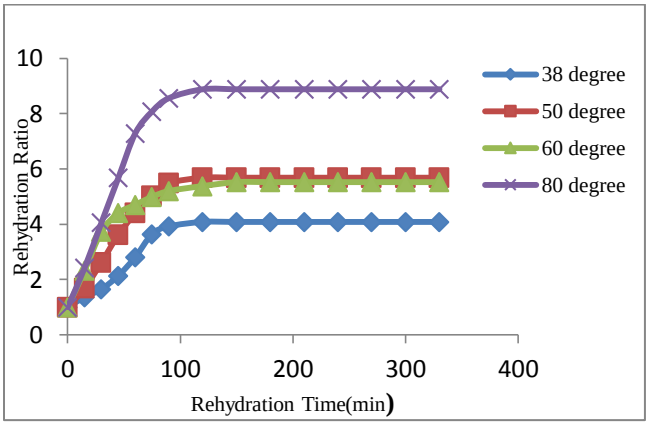

Figure 2.6: Rehydration kinetics of red Amaranth leaves at different temperatures for 60°C temperature dried processed sample

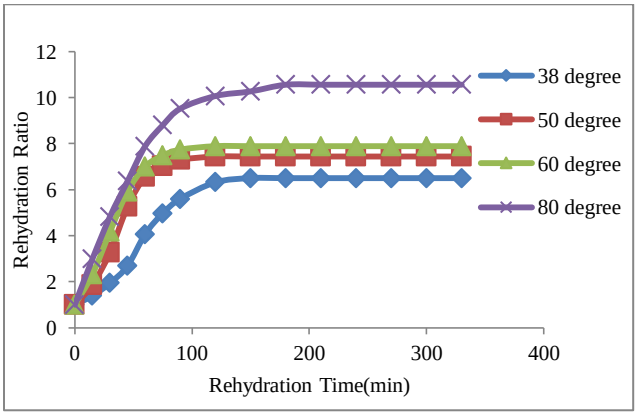

Figure 2.7: Rehydration kinetics of red Amaranth leaves at different temperatures for 80°C temperature dried control sample

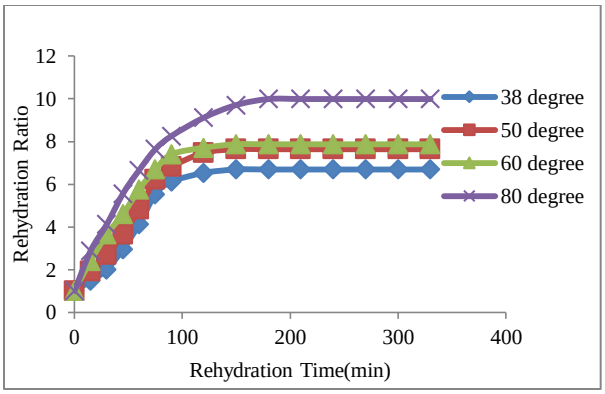

Figure 2.8: Rehydration kinetics of red Amaranth leaves at different temperatures for 80˚C temperature dried chlorinated sample

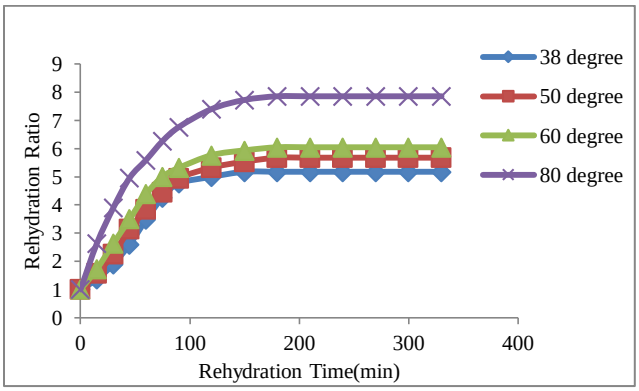

Figure 2.9: Rehydration kinetics of red Amaranth leaves at different temperatures for 80˚C temperature dried processed sample

Figure2.1, Figure2.2 and Figure2.3 show the rehydration kinetics of red amaranth leaves at four different temperatures(38˚C,50˚C,60˚C and 80˚C) for 50˚C dried control, chlorinated and processed sample respectively.Fig2.4,Fig2.5and Fig2.6 represent the rehydration kinetics of control, chlorinated and processed sample of red amaranth leaves dried at 60˚C and Fig2.7,Fig2.8 and Fig2.9 represents the same at 80˚C temperature dried sample. During rehydration, the water intake is more drastic during the first 33 min, then tapering off. Rehydration at 60˚C temperature shows the higher water absorbance and the chlorinated sample taken maximum amount of water and after rehydration it looks like the fresh one. Color, texture and the freshness are better than the other samples. So, the overall acceptance of this rehydrated sample is higher.

Table 2.1 shows the Peleg rate constant (K_1) and Peleg capacity constant (K_2) for every rehydration process for four different rehydration temperature and its graphical representation based on $t/ (M_0-M_e)$ versus time (t) ploted. The major advantage of the Peleg model is to predict equilibrium moisture content using short-time experimental data and selected for calculating the values of Peleg rate constant (K_1) and Peleg capacity constant (K_2) which affects the results we obtained (17). When the rehydration temperature is increased, the Peleg rate constant (K_1) and the Peleg capacity constant (K_2) both are consequently decreased, and this phenomenon effects the temperature, which is significant ($p \leq .05$) as increasing the temperature from 50˚C to 60˚C.

Table 2.1. Result's for peleg model for Red Amaranth leaves rehydration at 38˚C–80˚C temperature.

Sample	**Pretreatment**	**Rehydration Temperature**	**R^2 value**	**K_1Value**	**K_2 Value**
	Control	38˚C	0.961	0.773	3×10^{-3}
		50˚C	0.978	0.350	2.3×10^{-3}
		60˚C	0.977	0.319	1.9×10^{-3}
		80˚C	0.968	0.372	1.8×10^{-3}
50˚C temperature dried sample	Chlorinated	38˚C	0.897	1.152	2.7×10^{-3}
		50˚C	0.951	1.073	2.9×10^{-3}
		60˚C	0.996	0.095	1.2×10^{-3}
		80˚C	0.936	0.540	1.2×10^{-3}
	Processed	38˚C	0.842	2.651	1.8×10^{-3}
		50˚C	0.966	1.022	1.9×10^{-3}
		60˚C	0.990	0.482	2×10^{-3}
		80˚C	0.990	0.428	2.1×10^{-3}
	Control	38˚C	0.972	1.024	2.8×10^{-3}
		50˚C	0.973	0.784	1.7×10^{-3}

		60˚C	0.971	0.714	$1.6x\ 10^{-3}$
		80˚C	0.988	0.495	$1.4x\ 10^{-3}$
60˚C temperature dried sample	Chlorinated	38˚C	0.973	1.390	$2.8\ x\ 10^{-3}$
		50˚C	0.987	0.750	$2.6x\ 10^{-3}$
		60˚C	0.989	0.526	$2.6x\ 10^{-3}$
		80˚C	0.996	0.264	$2.4x\ 10^{-3}$
	Processed	38˚C	0.984	0.908	$3.5x\ 10^{-3}$
		50˚C	0.989	0.552	$2.5x\ 10^{-3}$
		60˚C	0.997	0.315	$2.7x\ 10^{-3}$
		80˚C	0.988	0.352	$1.6x\ 10^{-3}$
	Control	38˚C	0.957	1.366	$2.8x\ 10^{-3}$
		50˚C	0.985	0.557	$2.7x\ 10^{-3}$
		60˚C	0.992	0.408	$2.6x\ 10^{-3}$
		80˚C	0.990	0.487	$1.9x\ 10^{-3}$
80˚C temperature dried sample	Chlorinated	38˚C	0.959	1.313	$2.9x\ 10^{-3}$
		50˚C	0.974	0.986	$2.6x\ 10^{-3}$
		60˚C	0.989	0.649	$2.7x\ 10^{-3}$
		80˚C	0.987	0.68	$2.1x\ 10^{-3}$
	Processed	38˚C	0.978	1.034	$3.1x\ 10^{-3}$
		50˚C	0.985	0.903	$2.8x\ 10^{-3}$
		60˚C	0.988	0.742	$2.7x\ 10^{-3}$
		80˚C	0.993	0.506	$2.1x\ 10^{-3}$

2.4 Conclusion

The rehydration ratio is higher at 60°C temperature for dried chlorinated, processed and control samples. An increase in temperature above 60°C had an adverse effect on the final rehydration ratio value, and it is decreased with increasing temperature. It is indicated that during the rehydration process temperature-induced to change the product and the loss of solids.

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Chapter 3

Osmodehydration of Pineapple cuboid and its storage study

3.1.Introduction:

Pineapple (Ananas comosus) is the most commercially significant tropical fruit crops in the world. It is known as the "queen of fruits" due to its excellent flavour and taste [1]. After citrus and bananas, pineapple is the third most significant tropical fruit in the world [2]. A good amount of bromelin, a protein-digesting enzyme, citric acid, malic acid, and vitamins A and B are also present in mature fruit, which also includes 14% of sugar [3]. The ingredients in pineapple juice vary based on the region, time of year, harvesting method, and process. The fruit's flavour is refreshing because of the fruit's sugar and acid balance. The pineapple is a beautiful tropical fruit that offers a tonne of health advantages along with exceptional juiciness and bright tropical flavour. The significant amounts of calcium, potassium, vitamin C, carbohydrates, dietary fibre, water, and other minerals found in pineapple are helpful for the digestive system, aid in maintaining a healthy weight, and promote balanced nutrition [30]. 100 g of edible pineapple contains 50 Kcal (USDA nutrient database). According to research in "Alternative Therapies in Health and Medicine," pineapple can treat allergies and asthma and it reduces stress and relaxes the heart.

Recent years have seen a significant interest in the preservation of freshness of fruits and vegetables through the submersion of water-containing cellular components in an osmotic solution. Due to the solute gain and water loss, it causes the formation of intermediate moisture products with reduced water activity. The technique significantly decreases the chemical, physical, and biological activity that induce foods to decay, hence extending the storage stability of food stuffs. Phase change has been avoided in this process because moisture is removed from the product at ambient temperature through diffusion. Additionally, it enhances the nutritional value and sensory qualities of food goods and uses less energy than other drying methods. Osmotic agent, time and temperature, solute concentration, solution to sample ratio, agitation, and material structure are among the variables that might affect osmotic dehydration. Osmotic dehydration has recently been combined with a number of additional techniques, including pasteurization, microwave heating, high hydrostatic pressure, centrifugal force, vacuum, gamma irradiation, and blanching [31]. These methods have been used before, during, or after osmotic treatment to improve the efficiency of osmotic dehydration by enhancing mass transfer rate and cell membrane permeability. The drying period is shortened due to these combined activities,

which also reduce additional energy costs while enhancing the quality of fruits and vegetables during storage. Osmodried foods have gain great attaintion due to its potential benifits in food industry. By submerging plant tissues in a hypertonic solution, osmotic dehydration is frequently employed to remove some water from plant tissues. Osmotic lack of hydration is a conventional interaction applied to food dewatering. It prompts alluring items that are prepared to eat or can be applied as a pretreatment to the following system, for example, drying or freezing. Water is a principal constituent of food sources, which influences food security, microbial as well as compound. It is liable for the customer view of quite a large number organoleptic qualities like juiciness, elasticity, tenderness and texture. In food industry, food and food products are preserve by using dehydration process to remove the water from the raw materials. The bringing down of water action can be accomplished in two ways, either by the expansion of humectants or by the expulsion of solvent, for example, water. Now a days, researcher focused on the improvement of product quality of preserved food products. Osmotic dehydration is the cheapest and easiest method to obtain better food products by removing water at low temperature [4]. It has been broadly utilized as a pretreatment step in dehydration techniques since it can diminish generally the energy prerequisite for additional dehydration process [5]. To fulfill the developing market interest for products in a fresh like state, insignificant handling like osmotic lack of hydration will be progressively utilized. The dynamic exploration in the area of osmotic drying out of organic products is proceeding from one side of the planet to the other.

Pineapple is the most popular tropical fruits in India. The overall production of pineapple in India is grown across an area of 89 thousand ha, is 1,415.00 thousand tonnes. It is native to Central and South America. The cultivation of pineapple was initially begun in regions among Brazil and Paraguay, and afterward steadily spread over the entire tropical world. The all out region under pineapple development on the planet is 1.05 million hectare with creation around 25.81 million hectare [6]. The entire North-Eastern region of India, Tripura, Assam, Meghalaya and Manipur, the northern part of West Bengal, the coastal regions of Andhra Pradesh, Orissa, Kerala, Tamil Nadu, and Goa, as well as some areas of Maharashtra, Gujarat, and Karnataka are the best climate in India for the production of pineapple. India contributes more than 10% of the world's total pineapple production area, placing it in second place behind Nigeria (0.18 million hectare), with over 0.11 million hectares under pineapple cultivation. Costa Rica is the top producer (2.92 million tonnes) country in the world. India placed sixth position in production

with 1.74 million tones. In India, there are only three varieties that are widely cultivated: Giantkew, which is a large fruit with broad, flat eyes and a range of yellow to coppery yellow flesh colour (12–14 orbix); Queen, which is a small fruit with small, raised eyes and a deep golden yellow flesh colour (15–16 orbix); and Mauritius, which is a medium-sized fruit with a yellow and red colour. There are numerous local names in India, including Kaitachchakka (Malayalam), Keehom (Manipur), Ananus (Marathi), Annasahannu (Kannada), Anasipazham (Tamil), and Annasapandu (Telegu). Pineapple is more flavoured and testier when it grown in sunny land of northeast India. After receiving tropical rain then it packing and delivered to storage section. [7]. Fresh, cooked, juiced, and preserved pineapples are all consumed or served. This fruit is seasonal and extremely perishable should be used within four to five days of being harvested. Indians are typically eating ripe pineapple. The dried waste is used as animal feed after the juice has been extracted. Canned pineapple slices are most popular marketed product all over the world. Pineapple can be used to create pickles, fruit salads, squash, jelly, citric acid, vinegar, alcohol, and syrup [8]. In developing nations, up to 40% of agricultural waste is lost due to lack of storage and processing facilities and inadequate knowledge of processing techniques [9]. Osmotic dehydration is frequently used as a pre-treatment prior to further processing or to eliminate a portion of the water content of fruit in order to obtain a product of intermediate moisture [10,11]. Rehman (1990) studied the osmotic dehydration of pineapple [12].

Pineapple organic product is viewed as an exceptionally nutritious natural product since it contains an elevated degree of L-ascorbic acid, a characteristic cell reinforcement which might restrain the improvement of major clinical circumstances including coronary illness and certain malignant growths [26]. The organic product likewise contains phenolic compounds and β-carotene [13,14, 1516,17,26,27], which comprise normal wellsprings of cell reinforcements. Consequently data in regards to cancer prevention agents and cell reinforcement limit of "Phulae" and "Nanglae" pineapples is required to serve purchasers. The target of this study is to examine the physical and physicochemical qualities, including bioactive mixtures and cancer prevention agent limit of these two pineapple cultivars. The data acquired from this study will be helpful for advancing and expanding organic product utilization and their financial worth.

The purpose of this study is to investigate the impact of processing on physiochemical and biochemical properties of osmodehydrated pineapple (*Ananas comosus (L) Merr*.) cuboid and its storage stability.

3.2. Materials and Methods:

3.2.1. Preparation of osmo-dehydrated pineapple cube:

Raw pineapples were obtained from Jadavpur market near Jadavpur University, Kolkata, India. Well maturated and ripe pineapples were selected for this study. Cleaned thoroughly with distilled water to remove adhering, dust, foreign matter and wiped with a muslin cloth. The selected pineapples were peeled using stainless steel knife. The edible portion was cut into cubes after removing the core. Size of the pineapple cuboids are (5×2×2) mm^3.The treatments prior to osmo-dehydration consisted of-

a. Control sample(A_1)
b. Steam blanching (A_2)
c. Microwave heating(A_3)

3.2.2. Pretreatment:

3.2.2.1 Control: Pineapple cube without any treatment were considered as control

3.2.2.2 Microwave heating: Pineapple cuboids are put into microwave at 300W for 5 min

3.2.2.3Steam blanching: Steam blanching was done in a steamer for 5 min.

3.2.3. Osmo-dehydration Procedure:

Pineapple cubes were weighed approximately 15 g for every experiment and immersed in 100 ml sugar syrup and left undisturbed for 24 h at room temperature (28 ^{0}C) for osmosis. The samples were separately dipped in a sugar solution 30˚B by using hand refractometer (Erma Inc. Tokyo, Japan). The concentration of sugar syrup increased in 50˚B and 60˚B. Osmosis takes place and water goes from the fruit pieces to the syrup and a small amount of solute moves into the pineapple cuboids. After the osmosis process was complete, the fruit slices were removed out of

the osmotic solution and drained in order to eliminate of the sugar coating that had formed on their surface. Osmosed pineapple cuboids were spread uniformly over stainless steel trays and were kept in a conventional tray drier for dehydration with intermittent turning of cubes for quick drying. Pineapple cubes were dried at 40°C air temperature for overnight to get the desired (less than 5%) moisture content and product quality. Osmodehydrated cuboids were packed in 200 gauge polyethylene bags and stored under ambient condition for three months. Samples were analyzed for total phenol and antioxidant activity.

3.2.4Analytical parameters

3.2.4.1. Moisture Content

Moisture Content was calculated according to the method described by Ranganna (1986) and expressed as dry basis [18].

3.2.4.2. Determination of total polyphenolic content (TPC)

Total phenol content was determined using the Folin-ciocalteu's reagent as described by Singleton and Rossi(1965)[19].The sample extract (200µL)was mixed with 1.5ml of Folin-ciocalteu reagent (Previously diluted tenfold with distilled water). Allow to leave at room temperature for five minutes. Then 1.5ml sodium bicarbonate solution (60gm/L) was added to the mixture. Mixture was vortexed, covered and allowed to stand for 120 min in a dark place. Triplicate measurements were carried out and the absorbance was measured by spectrophotometer (Hitachi U- 2000) at 765nm against a blank containing all the reagents without the sample and plotted in a standard calibration curve of Gallic Acid. These results have been expressed as Gallic Acid Equivalents per gram of dry sample.

3.2.4.3. Determination of total antioxidant content (FRAP)

Antioxidant capacity of pineapple cuboids was calculated by FRAP (ferric reducing/ antioxidant power) assay at a wavelength of 593nm using a spectrophotometer by Benzie and strain as modified by pulido, (Benzie and strain, 1996) and expressed as mg of ascorbic acid/ 100gm of dry red amaranth leaves[20].

3.2.4.4.Determination of water loss (WL) and solid gain (SG)

The following equation was used to determine the WL and SG of osmotic dehydrated samples after they had been wiped with tissue paper [21]

$$WL = \frac{W_{wo} - W_w}{W_o} \times 100$$

$$SG = \frac{W_s - W_{so}}{W_o} \times 100$$

Where, WL and SG are water loss and solid gain in %, respectively.

Wwo is the initial water mass,

Ww is the mass of water at time t,

WS is the solid mass at time t,

Wso is the initial solid mass

3.2.4.5.Dehydration and rehydration ratios :

The ratio of the sample weight before drying to the sample weight after drying was used to calculate the dehydration ratio. While the weight of the rehydrated sample to that of the dehydrated sample was used to calculate the rehydration ratio. [22].

$$\text{Dehydration ratio} = W/W_D$$

$$\text{Rehydration ratio} = W_R/W_D$$

W is the sample's weight before drying, W_D is its weight after drying, and W_R is its weight after being rehydrated (g).

3.2.4.5.Organoleptic evaluation of osmotically dried pineapple cuboids

Descriptive sensory evaluation was carried out to determine the effect of osmo-drying on the quality parameters of osmo-dehydrated pineapple slices. A 6-member sensory panel of semi-trained judges assessed using a 9-point hedonic scale for colour, texture, flavour, taste,appearance and overall acceptability of the osmotic dehydrated pineapple cuboids[23]. The average value was calculated after attributes were rated for degree of liking on a 9-point hedonic scale (1 = highly detest, 9 = extremely like). A score of 5.5 or higher was acceptable. The average of all the sensory parameters was recorded.

3.3.Result and Discussion:

Table 3.1: Physicochemical properties of fresh pineapple

Characteristics	**Value**
Average weight(gm)	1500 gm
Pulp (%)	70
Peel (%)	50
Core (%)	17
Waste Index (%)	58
Total Soluble solids (˚B)	11
Moisture Content (%)	88
Vitamin C (% mg)	38
Total phenol Content (mg GAE /g)	51.1
Antioxidant Activities (percentage of inhibition)	62.4

3.3.1. Effect of mass transfer under various sugar syrup concentrations:

During the standardization process, the impact of varying syrup temperature and sugar concentration on mass transfer as a function of time was investigated. Fig 3.1, Fig 3.2 and Fig 3.3 represents the result graphically. The rate of mass transfer was higher initially, but as time went on, it steadily decreased. After dipping for three hours, the weight was consistently dropped, and then it declined slightly for all concentrations. The sample treated with 60° B sugar syrup at room temperature showed the greatest mass transfer, whereas samples treated with 30°

B and 50° B sugar syrup showed the least amount of mass transfer. Compared to the sample treated with 30° B and 50° B, they are significantly hampered.

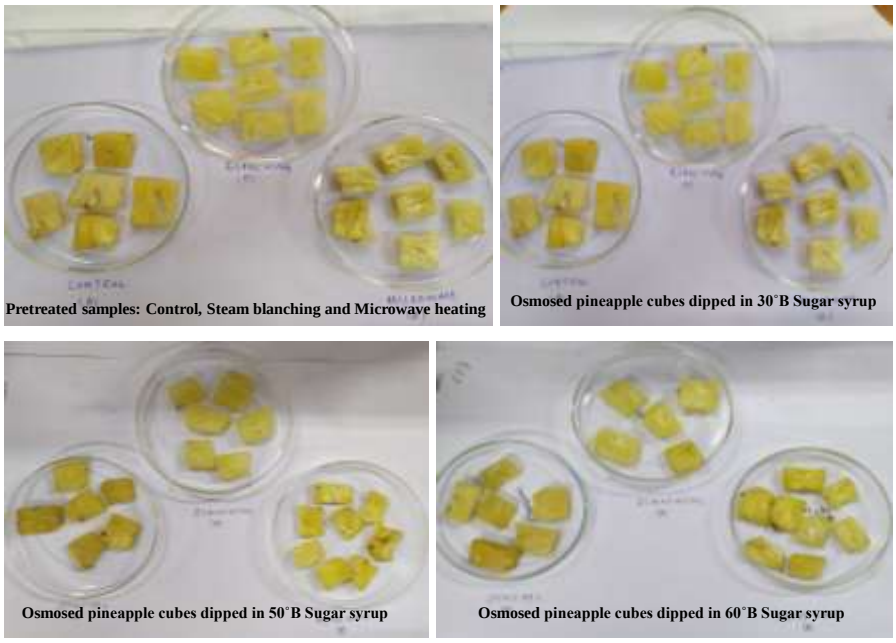

Figure3. 1: Pretreated samples and the samples are dipped in three different sugar syrups

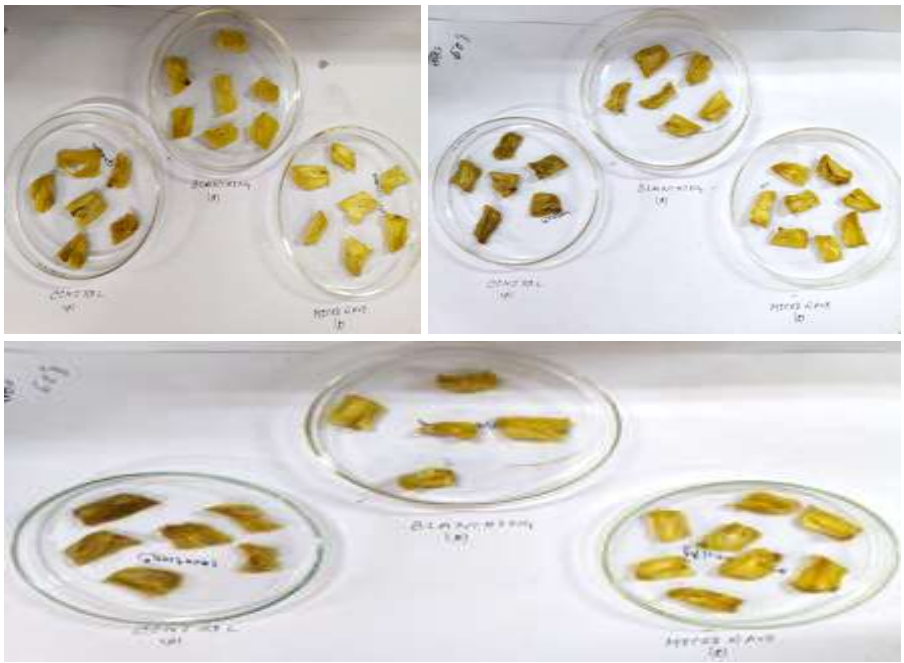

Figure 3.2: Osmodehydrated samples dried at 40˚C temperature

Figure 3.1 represents the pretreated samples and the samples were dipped in three different sugar syrup. Figure 3.2 represents the osmodehydrated samples dried at 40˚C.From these figures we see that after dipping in sugar syrup every pretreated sample look like same but after drying steam blanching and microwave heated samples are looks better. Microwave heated samples gives the better retention of vitamin C, it gives higher total phenol content and antioxidant activity. So the overall acceptability of osmodehydrated microwave heated pineapple cubes is higher than the steam blanched sample. Osmodehydrated control samples are not accepted due to bad color.

Table 3.2: Effect of sugar concentrations on osmotic dehydration of pineapple cuboid after drying, including weight loss (%), dry weight (g/100g), dehydration ratio (%), and rehydration ratio (%)

Sugar Concentration(˚B)	Treatment	Weight loss (%)gm	Dry weight (%)gm	Dehydration Ratio	Rehydration Ratio
30˚B	Control	69	30.75	3.25	3.43
	Steam blanching	60	34.06	2.94	2.97
	Microwave heating	65	40	2.5	2.63
50˚B	Control	80	20.43	4.89	4.8
	Steam blanching	76	25.49	3.92	2.61
	Microwave heating	75	24.14	4.14	4.86
60˚B	Control	75	24.89	4.01	3.88
	Steam blanching	66	21.30	4.69	4.31
	Microwave heating	79	33.84	2.95	2.84

3.3.2. Effect of temperature and sugar syrup concentration on mass transfer:

During the standardization process, the impact of various sugar concentrations and syrup temperatures on mass transfer as a function of time was investigated. Figures 3.1 through Figure 3.3 provide a visual representation of the results. The weight decreased steadily for the first three hours following dipping, and then it decreased slightly at all concentrations. After three hours of immersion, the weight was consistently lost, and it then gradually decreased for all concentrations. The rate of mass transfer was higher initially but steadily decreased with time. Minimum mass transfer was seen in samples treated with 30° Brix and 50° Brix sugar syrup,

while maximum mass transfer was reported in samples treated with 60° Brix at room temperature of sugar syrup. These are considerably inhibited as compared to samples that were treated with 30° and 50° brix.

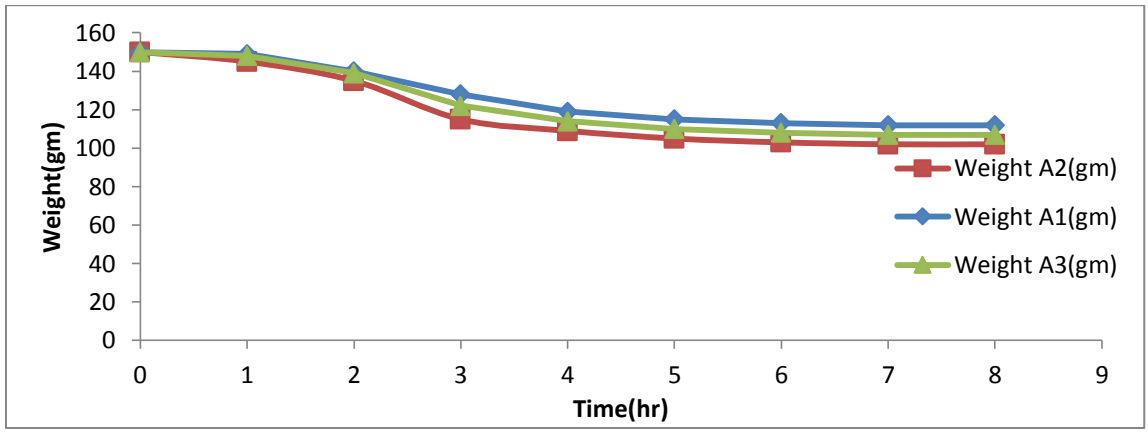

Figure 3.3: Effect of sugar syrup concentration30˚B on mass transfer at room temperature

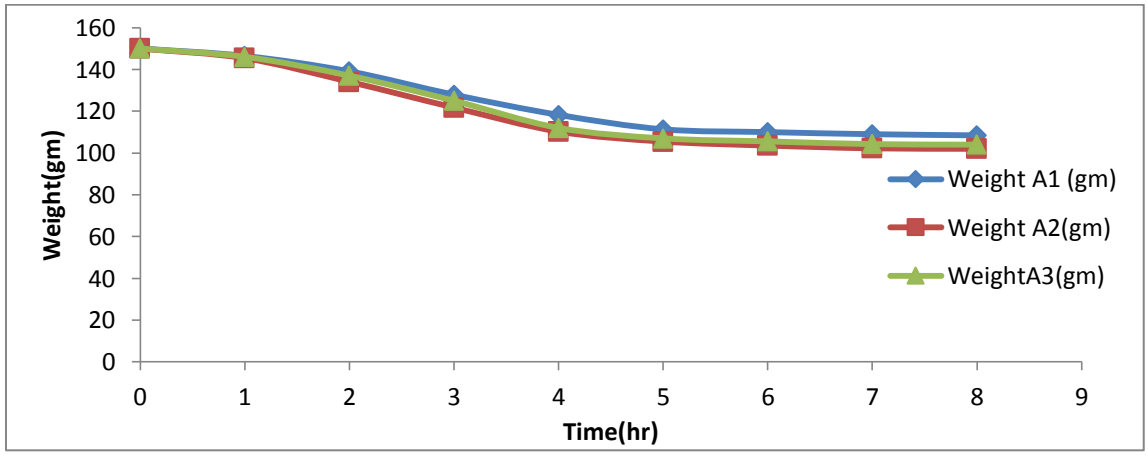

Figure 3.4: Effect of sugar syrup concentration50˚B on mass transfer at room temperature

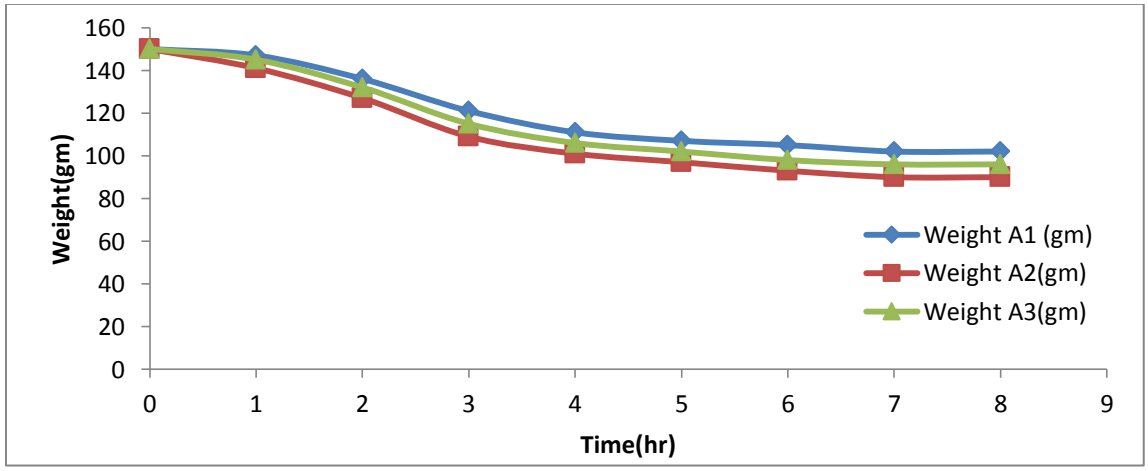

Figure3. 5: Effect of sugar syrup concentration60˚B on mass transfer at room temperature

Table 3.2 provides the findings on the impact of sugar syrup concentration on moisture content. After the sample was removed from the sugar syrup, its moisture content was calculated. It was discovered that as syrup concentration increased, the moisture content decreased. The highest reduction in moisture content was seen in a sample at 60° B. Table 3.3 shows that the moisture content percentage in the 60° B sample is lower than that in the other samples. As the concentration raised, the ascorbic acid content gradually reduced, probably as a result of ascorbic acid converting to dehydro-ascorbic acid.

Table 3.3: Effect of moisture content on the concentration of sugar syrup

Concentration of sugar solution	Sample	Moisture content (%)
30˚B	Control	80
	Steam blanching	76
	Microwave heating	75
50˚B	Control	75
	Steam blanching	66
	Microwave heating	79
60˚B	Control	69
	Steam blanching	60
	Microwave heating	65

Table3.4: Effect of sugar syrup concentration on Vitamin C, total phenol content and antioxidant content of pineapple cuboids

Sample	Sugar syrup concentration	Vitamin C (mg/g)	Total phenol content (mg GAE/g)	Antioxidant activity
Control(A1)	30˚B	18.5±0.4	48.12±0.3	57.4±0.2
	50˚B	12.03±0.08	35.5±0.1	48.3±0.7
	60˚B	5±0.04	27.6±0.4	33.3±0.1
Steam blanching(A3)	30˚B	15.6±0.05	49.5±0.3	59.8±0.3
	50˚B	9.67±0.9	38.7±0.5	50.43±0.2
	60˚B	2.7±0.2	30.5±0.8	38.56±0.6

Microwave heating(A2)	30˚B 50˚B 60˚B	17±0.3 10±0.7 3.8±0.4	49.67±0.3 39.5±0.8 39.9±0.3	57.1±.3 49.2±0.46 48.14±0.5
Fresh		21.5	51.1±0.2	62.4±0.2

3.3.3. Sensory evaluation:

Table 3.4 represents the results of a 9-point hedonic scale analysis of osmotically dehydrated pineapple cuboids for several food quality parameters. It is discovered that the sample that was dipped in a 60° Brix solution and dried out performs noticeably better in terms of colour, flavor and texture than other samples. When the sample was submerged in a 60° Brix sugar syrup and then dried, the texture was superior. Over the other samples, the sample that was dried after being dipped in a 50° Brix solution has a better color,taste and flavour.

3.3.4. Osmotically treated pineapple cuboids:

Table 3.5: Osmotically treated pineapple cuboids after drying

Concentration of sugar syrup	Sample	Color	Taste	Texture	Flavour	Mouth feel	Overall acceptability
30˚B	Control Sample	7.9	7.6	7.8	7.9	7.6	7.76
	Steam Blanching	7.8	7.7	7.9	7.6	7.9	7.78
	Microwave Heating	7.9	7.8	7.9	7.9	7.8	7.86
50˚B	Control Sample	7.9	7.5	8.0	7.5	7.8	7.74
	Steam Blanching	8.1	7.6	8.0	8.0	8.0	7.94
	Microwave Heating	8.2	8.0	8.5	8.2	8.5	8.28
60˚B	Control Sample	7.8	7.6	7.5	7.6	7.8	7.66
	Steam Blanching	8.3	8.0	8.5	8.0	8.5	8.28
	Microwave Heating	8.5	8.2	8.7	8.2	9.0	8.52
Mean		8.04	7.77	8.09	7.87	8.1	7.98

STDEV		0.23	0.23	0.12	.22	.43	.28

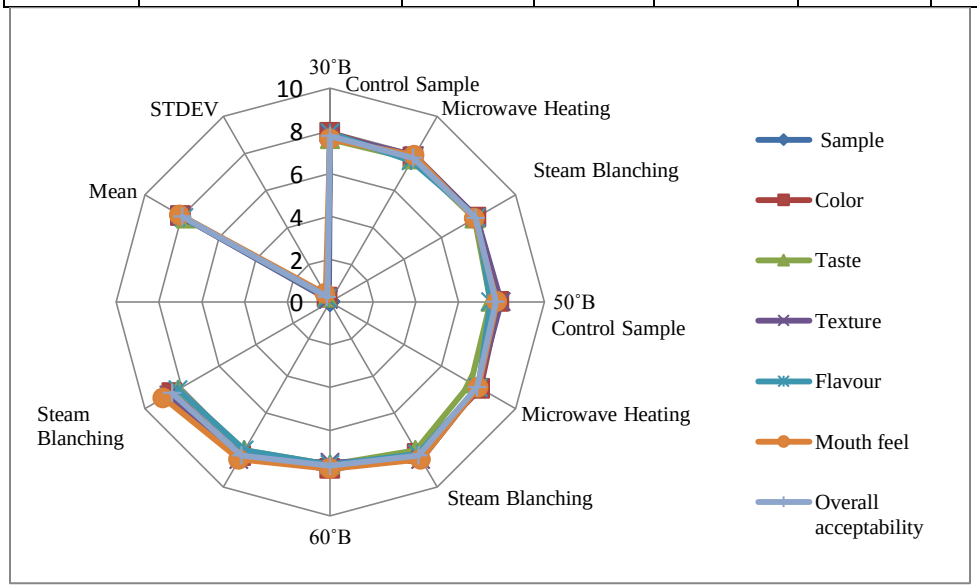

Figure 3.6: Sensory analysis of Pre-treated osmodehydrated Pineapple cuboids

3.3.5.Storage study of Pre-treated pineapple cuboids:

Table 3.6: Sensory analysis of pineapple cuboids during storage:

Quality Attributes	**Control Sample**				**Steam Blanching**				**Microwave Heating**			
	Initial	1 month	2 month	3 month	Initial	1 month	2 month	3 month	Initial	1 month	2 month	3 month
Color	7.8	7.6	7.1	5.4	8.3	8.2	8.0	7.5	8.5	8.3	8.2	7.8
Taste	7.6	7.5	7.0	5.6	8.0	8.0	7.8	7.4	8.2	8.1	8.0	7.5
Texture	7.5	7.3	7.1	5.8	8.5	8.3	8.0	7.1	8.7	8.5	8.3	7.6
Flavour	7.6	7.5	7.1	5.5	8.0	8.0	8.0	7.3	8.2	8.1	8.0	7.5
Mouth Feel	7.8	7.6	7.2	5.6	8.5	8.4	8.2	7.5	9.0	8.6	8.2	7.8
Overall Acceptability	7.66	7.5	7.1	5.58	8.28	8.18	8.0	7.36	8.52	8.32	8.14	7.64

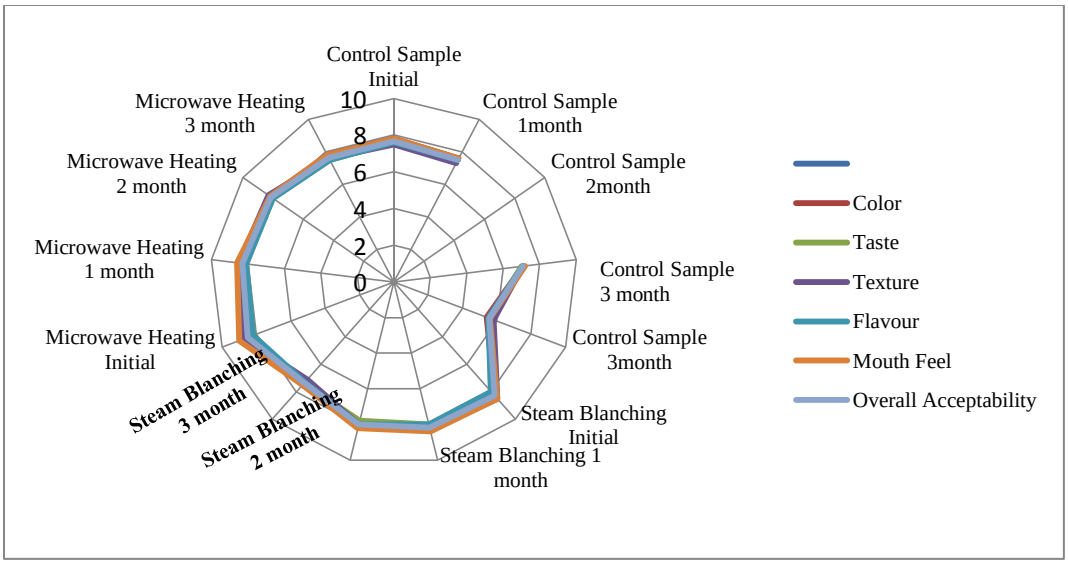

Figure 3.7: Descriptive analysis on Sensory score of pre-treated samples dipped in 60˚B sugar syrup

Table3.7: Effect of sugar syrup concentration on TSS, moisture content, vitamin C, total phenol and antioxidant activity of osmo-dehydrated pineapple cuboids during storage

Samples	Vitamin-C				Total phenol Content				Antioxidant Activity			
	Initial	1month	2month	3month	Initial	1month	2month	3month	Initial	1month	2month	3month
Control	5±0.02	2.67±0.05	0.8±0.02	0.4±0.06	27.6±0.1	18.6±0.2	11.32±0.3	8.7±0.45	33.3±0.1	17.04±0.02	9.07±0.06	4.06±0.05
Steam Blanching	2.7±0.02	2.5±0.4	1.8±0.05	1.5±0.02	30.4±0.8	29.89±0.034	23.04±0.05	16.07±0.02	38.2±0.5	35.02±0.3	28.4±0.2	21.5±0.4
Microwave Heating	3.8±0.47	3.5±0.05	3.1±0.023	2.5±0.04	39.8±0.3	36.5±0.07	31.7±0.02	27.6±0.03	48.1±0.3	45.5±0.03	39.8±0.04	30.06±0.02

3.4.Conclusion:

The kinetic study of osmodehydration process shows that when concentration of sugar syrup increased then the water loss and solid gain is increased. Pineapple cubes were osmodehydrated by using 60˚B sugar syrup concentration gives the better vitamin C retention , total phenol content is high better than the other. Also osmodried pineapple cube dipped in 60˚B gives the better antioxidant activity. Color, flavour, texture and the overall acceptability is high and stored for 3months at ambient condition without any adverse effect on the quality.

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Chapter 4

Modified atmospheric packaging of red amaranth leaves

4.1.Introduction:

Natural antioxidants, especially in fresh fruits and vegetables grown from the ground have acquired expanding interest among consumers and scientists on the grounds that epidemiological examinations have demonstrated that successive utilization of regular cancer prevention agents is related with a reduced risk of heart disease [2]. Rent there are three different types of antioxidant present naturally in fruits and vegetables vitamins, phenolics, and carotenoids—are linked to the protective properties of these compounds. Carotenoids are known as lipophilic antioxidants, whereas vitamin C and phenolics are regarded as antioxidants with hydrophilic in nature [3]. Modified atmosphere packaging (MAP) of fresh fruits and vegetables is based on modifying the levels of O_2 and CO_2 without addition of any preservative .The desired atmosphere can reduce the respiration rate, and ethylene production, physiological changes. It is highly advantageous for transportation of agricultural commodities with minimum loss. Presence of considerable amount of vitamin C, β-carotene, anthocyanin and antioxidant makes red amaranth leaves nutritionally rich leafy vegetables [4, 5, 6].This study will help in increasing the self life, utilization of leafy vegetables. Response surface methodology is a group of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing process. RSM requires lesser number of trial experiments to get relation between different parameters.Thus requiring less time effort.RSM has been widely applied for optimizing processes in the food industry. RSM has used for optimization of modified atmospheric packaging of red amaranth leaves. The objectives of this work was to study the effect of modified atmospheric packaging parameters on nutritional quality responses and also to optimize these parameters for developing an effective modified atmospheric packaging process to obtain higher quality final product.

4.2. Materials and methods

4.2.1. Moisture Content

Moisture content was determined by the method of Ranganna [8].10g sample was weighed accurately in a dry petridish and dried at 105˚C until constant weight. The moisture content was determined using the following equation-

$$\text{Moisture Content (\%)} = \left(\frac{A-B}{B}\right)$$

Where, A = the weight of the sample

B = the weight of the dry sample

4.2.2. Determination of total polyphenolic content (TPC): Described in Chapter 3[9]

4.2.3. Determination of total antioxidant content (FRAP): Described in Chapter 3[10]

4.2.4. Sample Preparation:

Undamaged matured fresh leaves of red amaranth were purchased in Jadavpur market near Jadavpur University area. Properly clean it in tap water for removing foreign matters like mud, dirt, chaff and immature leaves. The surface moisture was removed by using muslin cloth and after that it air dried at room temperature.

4.2.5. Packaging Material:

Low density polyethylene (LDPE) pouch was used for packaging material, the thickness of the package is 0.1mm and the package size is $0.022m^2$.

4.2.6. Optimization of freshness of red amaranth leaves using Box-Behnken Response surface methodology:

A statistical approach was made for the self-life extension of minimally processed red amaranth leaves. Box-Behnken design was used to analyze the conditions of modified atmospheric packaging which are dependent on time, temperature and the ratio between the size of the packet and the weight of the leaves. The experimental responses against independent variables are total phenol content, antioxidant activity and color. In this stage the preliminary study was to statistically optimize the range of experimental variables utilizing RSM. Optimized values of

design variables by OFAT were used as the Box-Behnken design of RSM. Table 4.2 represents a 17 preliminary test plan, where each factor was tried in three distinctive coded levels: low (-1), middle (0) and high (+1). The coded values correspond for the factors are shown in 4.1. For factual estimation, the correlation between the software utilized values and genuine values are depicted as follows:

$$X_i = (X_i - X_0) / \Delta X \quad \text{.....(i)}$$

Where X_i is dimensionless denoted value of the variable X_i , X_0 is real esteem of the X_i at the centre point; and ΔX is step change of factors. The optimization involved estimation of the coefficient in numerical model and foreseeing the reaction and really taking a look at the sufficiency of the model. A quadratic polynomial model can be used to represents the function in the range at interest as:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ii} X_i^2 \quad \text{.....(ii)}$$

Where, Y is the predicted response, β is constant coefficient, β_i is linear coefficients, β_{ij} is interaction coefficient, β_{ii} is quadratic coefficient and X, X are the denoted esteems of the independent process factors, and e is residual inaccuracy. The response surface design assists with examining the reaction above the whole factors space and to recognize the locale where it comes to its ideal worth. Reaction surface plot can give data about the blend af cycle's factors which gives the best reaction [11]

Table 4.1: The denoted level of varied conditions selected for these experiments

Independent variable	**Units**	**Coded**	**Coded variable level**		
			-1	**0**	**+1**
Time	Day	A	10	12	14
Temperature	Degree Celsius	B	18	20	22
S/W Ratio	Unitless	C	0.05	0.07	0.09

4.2.7. Statistical analysis:

'F' test was done to determine the statistical significance of this equation. The quality was checked by coefficient of determination R^2 value of a polynomial model. A repeatedly searches

are there for a good combination between factor levels used to optimization process to comparably assure each response and factors requirements too. Mathematical and graphical enhancement techniques were utilized in this investigation by choosing the ideal objectives for each factor and reaction was utilized in this examination. All the statistical analyses from experimental design to optimization were executed with statistical software Design Expert (Version 12.0; STATEASE INC.; Minneapolis, MN,USA).

4.3. Results and Discussion:

4.3.1. Optimization of environmental parameters for extension of selflife of red amaranth leaves through Box-Behnken Response surface Methodology:

The optimal level of the key factors and the effect of their interactions on modified atmospheric packaging of red amaranth leaves were explored by the Box-Behnken Response surface Methodology. Experimental design and results are shown in Table.

Table 4.2: Box-Behnken experiments design matrix with experimental variables and its responses for selflife extension of red amaranth leaves

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A:Time	B:Temperature	C:S/W Ratio	Total Phenol Content	Antioxidant Activity	Color
		Day	Degree Celcius	Unitless	mg/100gm of FAE	%Inhibition	Unitless
15	1	12	20	0.07	140.37	31.38	150.45
16	2	12	20	0.07	140.37	31.38	150.45
3	3	10	22	0.07	82.32	23.45	43.78
5	4	10	20	0.05	136.76	30.13	101.56
4	5	14	22	0.07	15.76	0.23	23.05
8	6	14	20	0.09	32.79	0.84	25
9	7	12	18	0.05	119.44	26.76	130.87
6	8	14	20	0.05	40.59	5.39	30.02
17	9	12	20	0.07	140.37	31.38	150.45
14	10	12	20	0.07	140.37	31.38	150.45
2	11	14	18	0.07	20.78	2.64	40.08

10	12	12	22	0.05	50.13	5.76	79.89
12	13	12	22	0.09	30.63	6.56	30.06
11	14	12	18	0.09	98.13	20.58	90.24
13	15	12	20	0.07	140.37	31.38	150.45
7	16	10	20	0.09	131.46	29.68	31.34
1	17	10	18	0.07	135.37	32.16	80.45

Modified atmospheric packaging of red amaranth leaves was described by the following established second order polynomial equation put in multiple regression analysis on the experimental data:

Equation in expression of coded factors-

Total Phenol Content (TPC)

Y_1 =-4425.86250+115.54187A+389.15562B+7314.68750C+3.00188AB-15.62500AC+11.31250BC-8.24937A^2-10.95375B^2 -54931.25000C^2

Antioxidant activity (FRAP)

Y_2 = -972.60562+32.76875A+81.11250B+1920.75000C+0.393750AB-25.62500AC+43.62500BC-1.89562A^2-2.29437B^2 -18218.75000C^2

Color

Y_3 =-5224.41937+356.42375A+318.11625B+7044.75000C+1.22750AB+407.50000AC-57.50000BC-17.42437A^2 -8.47812B^2 -84431.25000C^2

Making predictions about the response for specific levels of each variable can be performed using the equation expressed in terms of actual factors and the levels for each factor should be stated in their original units. The coefficients of this equation have been scaled to take into account the units of each factor, and the intercept is not located in the middle of the design space, therefore it should not be used to estimation the relative importance of each variable.

Where Y_1, Y_2, and Y_3 were the predicted yields of total phenol content (TPC), Antioxidant activity (FRAP) and color respectively. A,B,C were the coded values of time, temperature and s/w ratio correspondingly. The coefficient of determination R^2 was used to fixed out the decacy of fit of the model equation. Statistical suggestion of that equation was future studied by Fisher's test. The R^2 was measured by the degree of fit. The R^2 was conceivately enhanced the empirical representation fits of the real values [11].

The model F-value suggested that the model is dependable with the total phenol content, antioxidant activity and color. Values were 34.46, 28.65 and 70.88. A chance of arising was 0.01% of Model-F-value for the noise. The p-esteems are utilized as a device to check meaning of every factor, which likewise show the connection strength between every independent factor. P-values less than 0.05 in this study indicate the model expressions are considerable. In this case all the coded values are important model expressions. The coefficient of variation R^2, from the equation 3,4 and 5 were found 0.9779,0.9736 and 0.9891 indicated excellent contormity connecting observed and predicted results and suggested that the numerical model is trustworthy for selflife extension of modified atmospheric packaged red amaranth leaves in this study. Simultaneously the lack of fit measurement, which is utilized to test the ampleness of the model, show that the P-value of time, temperature and S/W ratio were not significant. No irregularity was seen from the findings of residuals. To test the importance of the model's second order polynomial equation for experimental data ANOVA was supervised (Table 4.2). From ANOVA data it could be accomplished that the predicted R^2 is in coherent conformity with the adjusted R^2. Using this statistical software (Design expert version 12.0.1.0) desirability and regression analysis was done. The independent variable optimized with assist of desirable task criteria existing in this software plan was to boost total phenol content, antioxidant activity and color turn over preserving the factors in their applicable investigational scale. So utilizing this desirability of rule was hold on to highest i.e.0.9495, 0.9396 and 0.9752. Desirablity (d=1) if activity $\geq$ high value,(d=0) if enzyme activity < low value, $0 \leq d \leq 1$ between low and high activity [12]. Thus, it can be concluded that the model was statistically sound. The contour plots shown depict the relationship between two variables by maintaining the other variables of their zero value for extension of selflife of red amaranth leaves. Model of response plane indicate the character and scope of the interaction between different factors. The contours plots' circular shapes typically indicate less significant or negligible interactions, while their elliptical shapes

indicate more significant interactions. The contour plots also show that the surface constrained in the tiny oval in the graph best represents the most extreme anticipated value. [39].

Table 4.3: ANOVA for quadratic model table for second order polynomial curve of Total phenol content

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	39622.36	9	4402.48	34.46	< 0.0001	significant
A-Time	17671.06	1	17671.06	138.30	< 0.0001	
B-Temperature	4747.28	1	4747.28	37.15	0.0005	
C-S/W Ratio	363.29	1	363.29	2.84	0.1356	
AB	576.72	1	576.72	4.51	0.0712	
AC	1.56	1	1.56	0.0122	0.9151	
BC	0.8190	1	0.8190	0.0064	0.9384	
A^2	4584.57	1	4584.57	35.88	0.0005	
B^2	8083.18	1	8083.18	63.26	< 0.0001	
C^2	2032.80	1	2032.80	15.91	0.0053	
Residual	894.41	7	127.77			
Lack of Fit	894.41	3	298.14			
Pure Error	0.0000	4	0.0000			
Cor Total	40516.76	16				

Table 4.4: ANOVA for quadratic model table for second order polynomial curve of Antioxidant activity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2634.26	9	292.70	28.65	0.0001	Significant
A-Time	1412.99	1	1412.99	138.32	< 0.0001	
B-Temperature	266.11	1	266.11	26.05	0.0014	
C-S/W Ratio	13.47	1	13.47	1.32	0.2886	
AB	9.92	1	9.92	0.9713	0.3572	

AC	4.20	1	4.20	0.4114	0.5417	
BC	12.18	1	12.18	1.19	0.3110	
A^2	242.08	1	242.08	23.70	0.0018	
B^2	354.64	1	354.64	34.72	0.0006	
C^2	223.61	1	223.61	21.89	0.0023	
Residual	71.51	7	10.22			
Lack of Fit	71.51	3	23.84			
Pure Error	0.0000	4	0.0000			
Cor Total	2705.77	16				

Table4.5: ANOVA for quadratic model table for second order polynomial curve of Color

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	43458.65	9	4828.74	70.88	< 0.0001	significant
A-Time	2414.43	1	2414.43	35.44	0.0006	
B-Temperature	3397.35	1	3397.35	49.87	0.0002	
C-S/W Ratio	3432.06	1	3432.06	50.38	0.0002	
AB	96.43	1	96.43	1.42	0.2729	
AC	1062.76	1	1062.76	15.60	0.0055	
BC	21.16	1	21.16	0.3106	0.5947	
A^2	20453.65	1	20453.65	300.25	< 0.0001	
B^2	4842.35	1	4842.35	71.08	< 0.0001	
C^2	4802.45	1	4802.45	70.50	< 0.0001	
Residual	476.86	7	68.12			
Lack of Fit	476.86	3	158.95			
Pure Error	0.0000	4	0.0000			
Cor Total	43935.51	16				

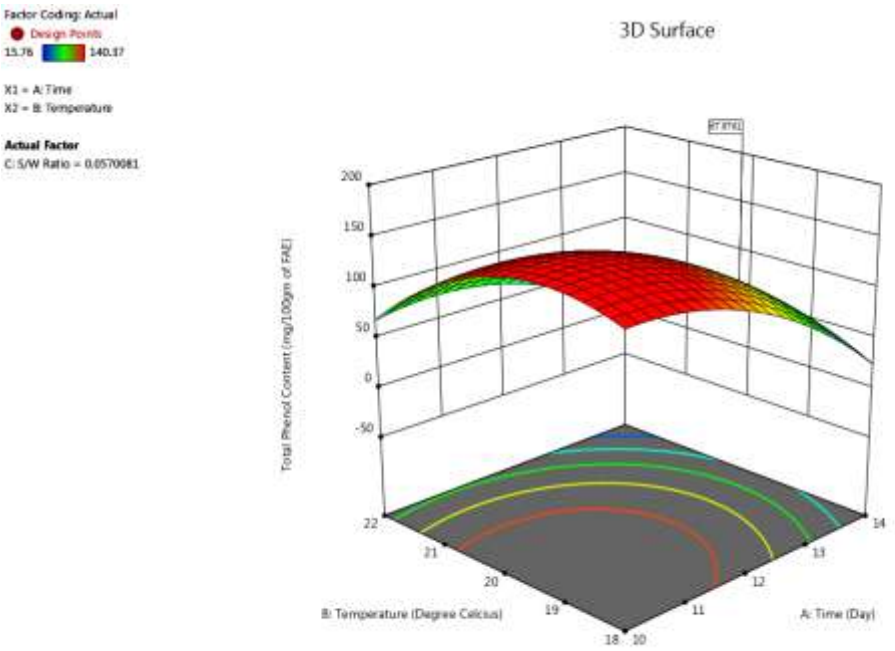

Figure 4.1: 3D response surface plot of interactive effect of total phenol content on Time and temperature

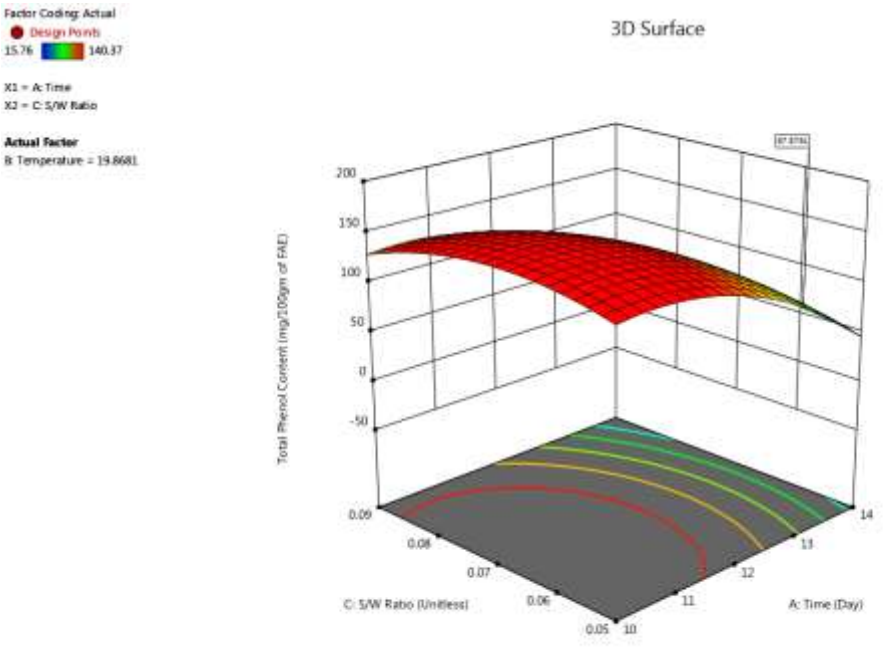

Figure 4.2: 3D response surface plot of interactive effect of total phenol content on Time and S/W ratio

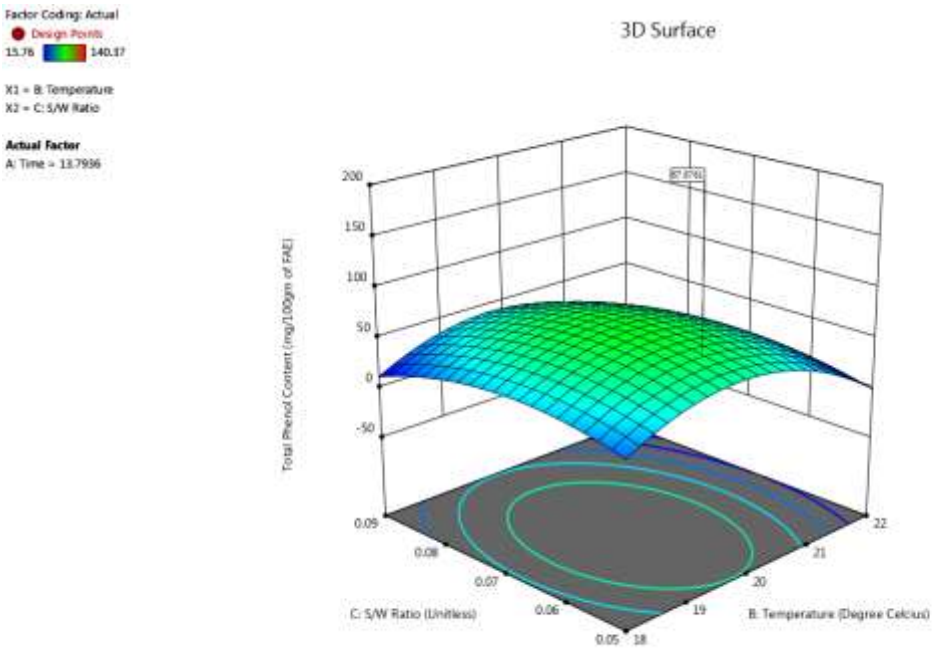

Figure 4.3: 3D response surface plot of interactive effect of total phenol content on Temperature and S/W ratio

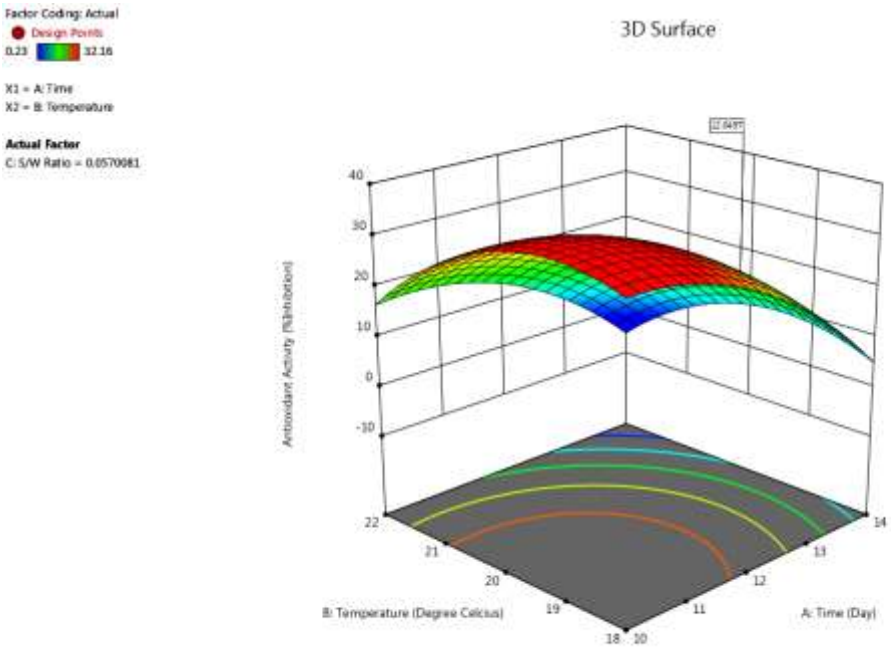

Figure 4.4: 3D response surface plot of interactive effect of antioxidant activity on time and temperature

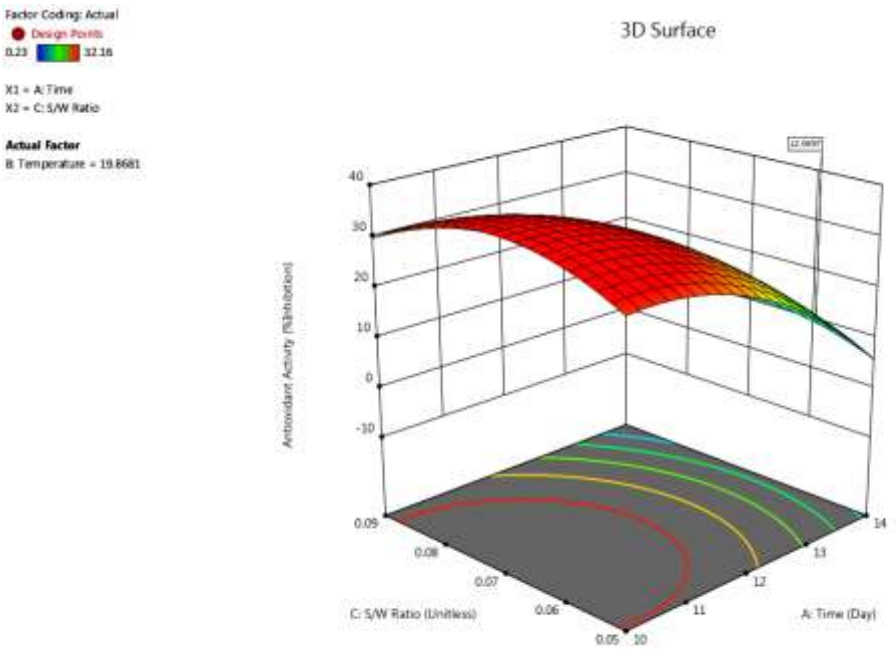

Figure 4.5: 3D response surface plot of interactive effect of antioxidant activity on time and S/W ratio

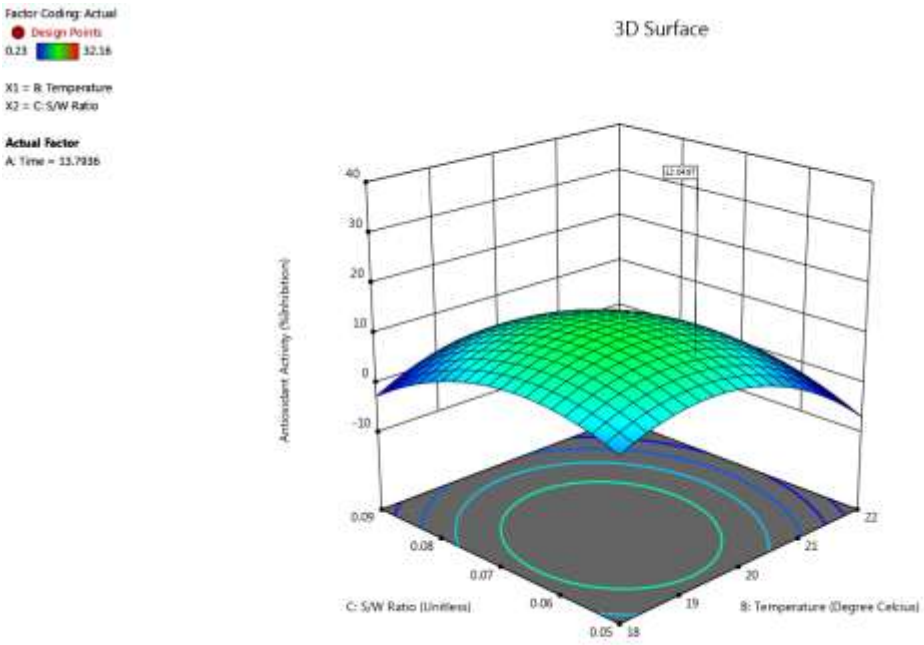

Figure 4.6: 3D response surface plot of interactive effect of antioxidant activity on temperature and S/W ratio

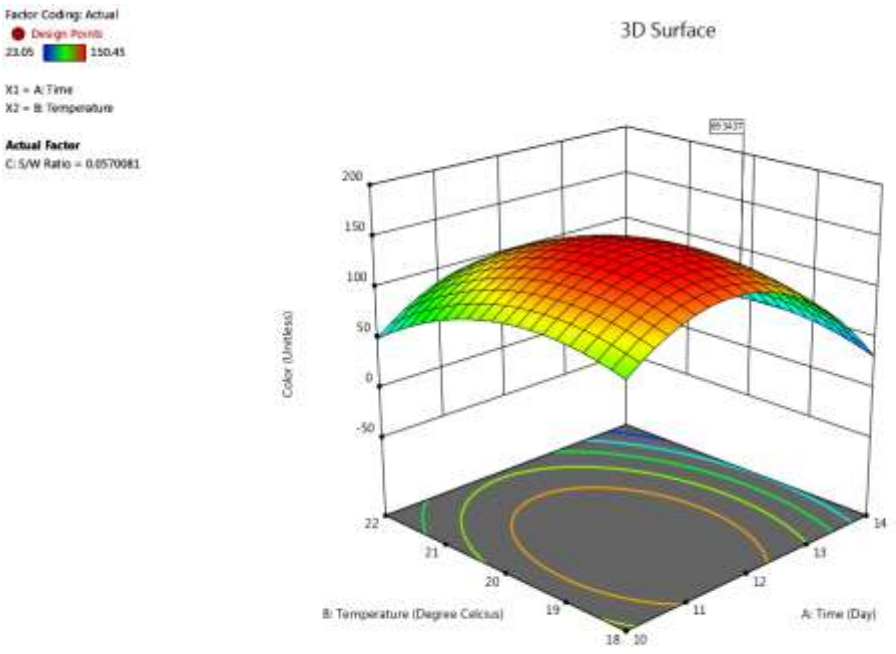

Figure 4.7: 3D response surface plot of interactive effect of color on time and temperature

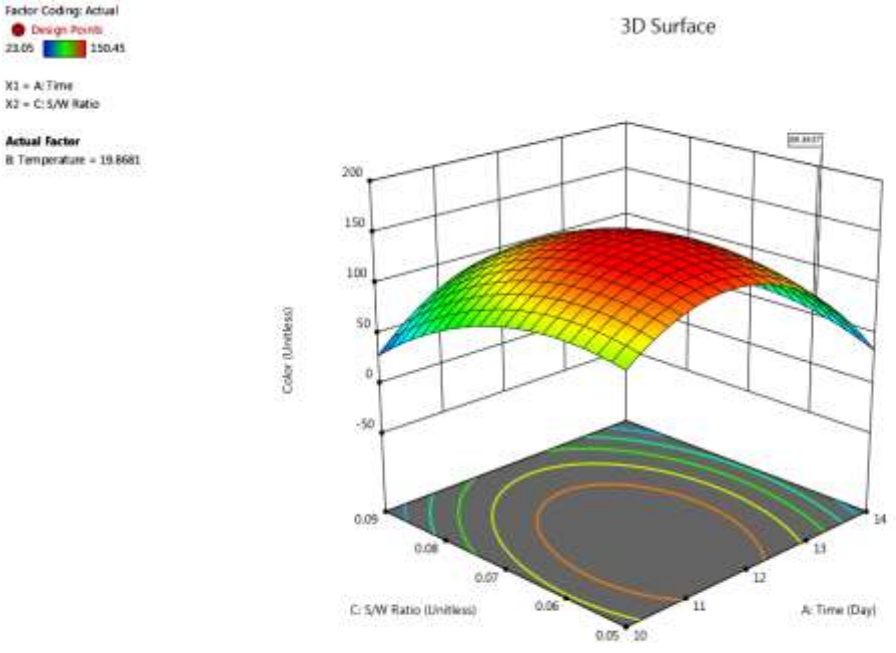

Figure 4.8: 3D response surface plot of interactive effect of color on time and S/W ratio

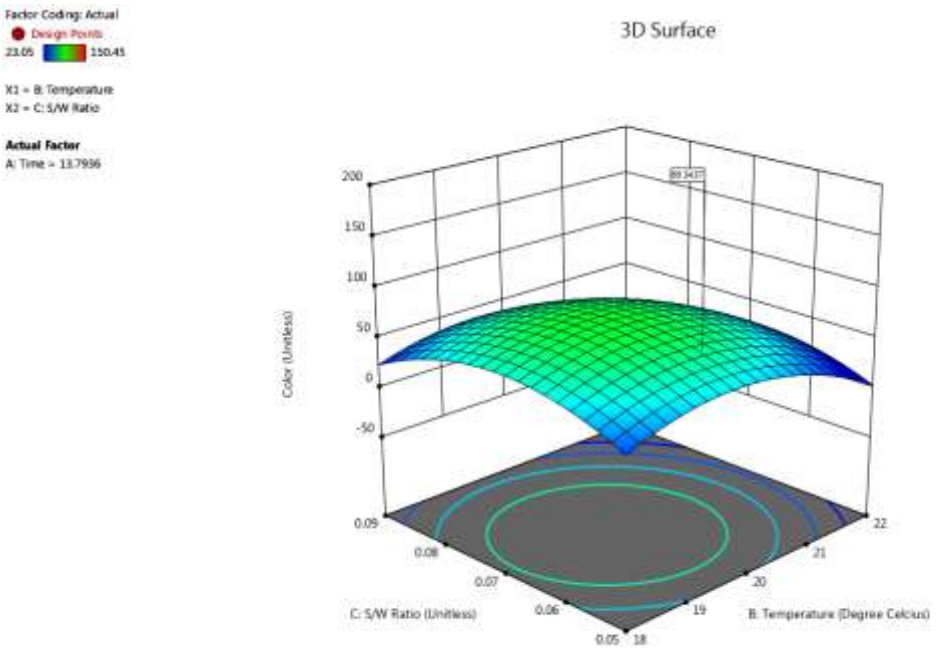

Figure 4.9: 3D response surface plot of interactive effect of antioxidant activity on temperature and S/W ratio

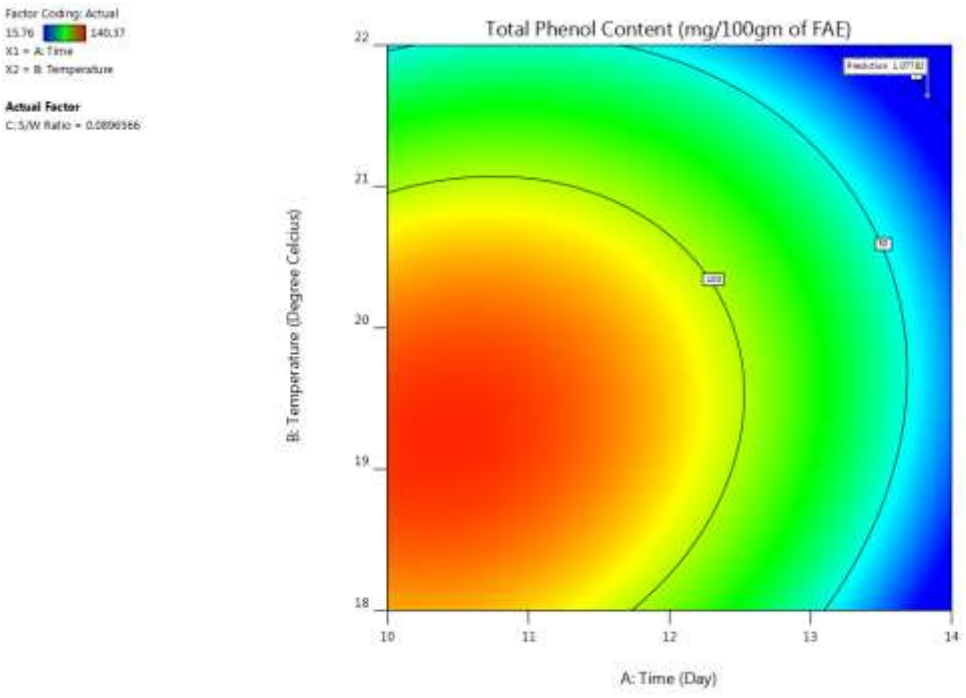

Figure 4.10: Contour plot of interactive effect of total phenol content on time and temperature on stability of fresh product

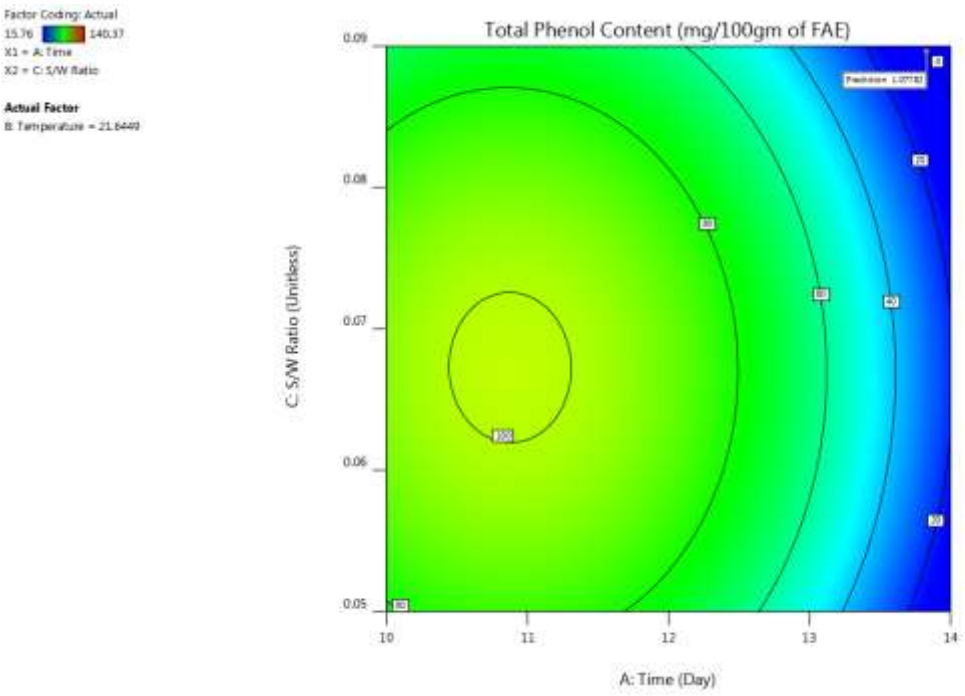

Figure 4.11: Contour plot of interactive effect of total phenol content on time and s/w ratio on stability of fresh product

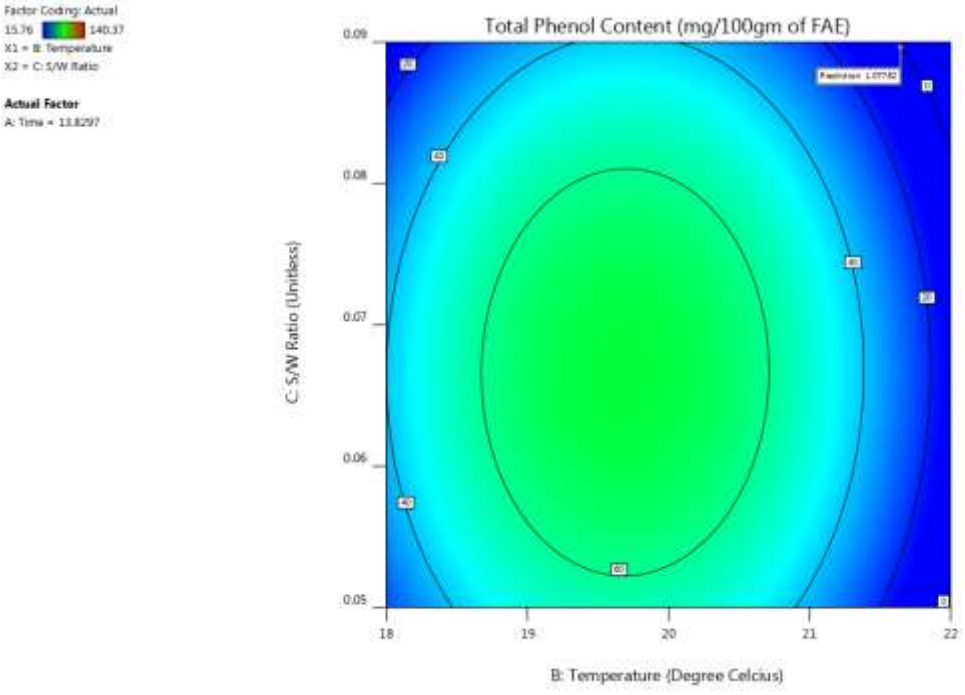

Figure 4.12 : Contour plot of interactive effect of total phenol content on temperature and s/w ratio on stability of fresh product

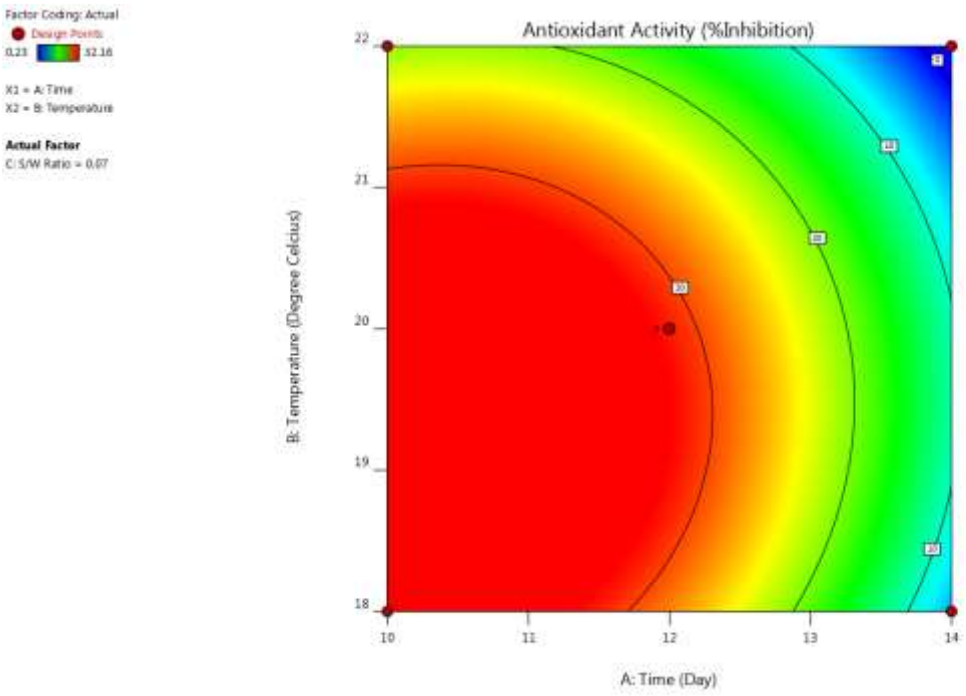

Figure 4.13: Contour plot of interactive effect of antioxidant activity on time and temperature on stability of fresh product

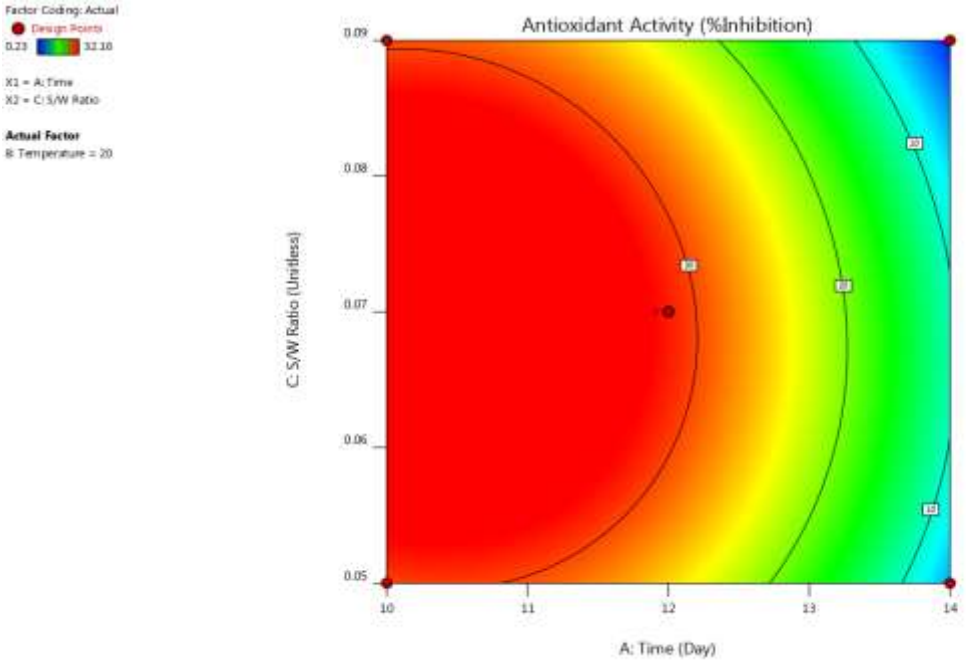

Figure4.14: Contour plot of interactive effect of antioxidant activity on time and s/w ratio on stability of fresh product

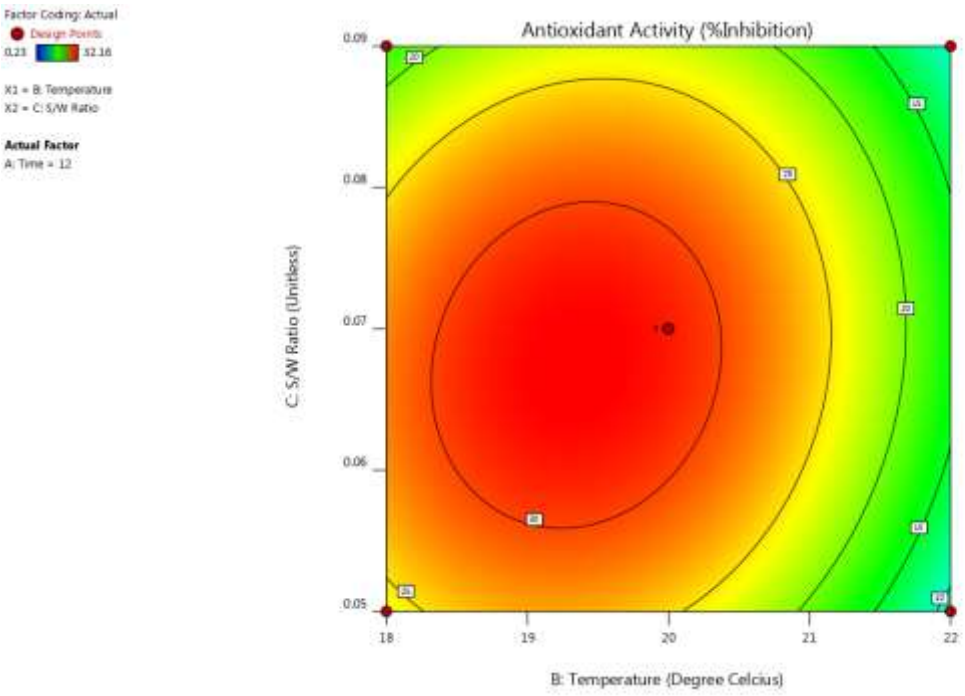

Figure 4.15 : Contour plot of interactive effect of antioxidant activity on temperature and s/w ratio on stability of fresh product

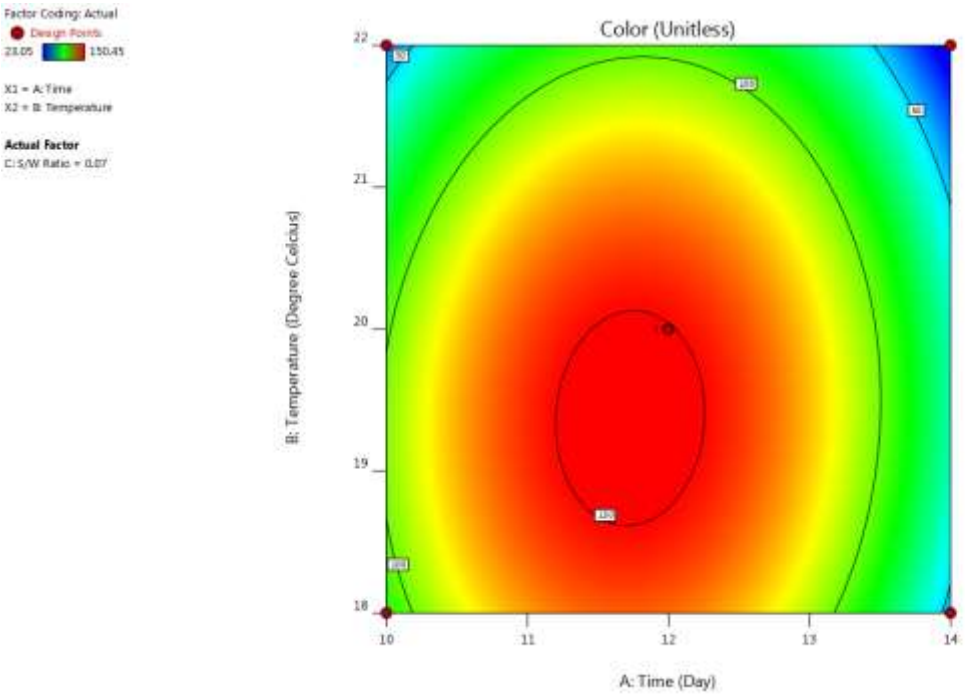

Figure 4.16: Contour plot of interactive effect of color on time and temperature on stability of fresh product

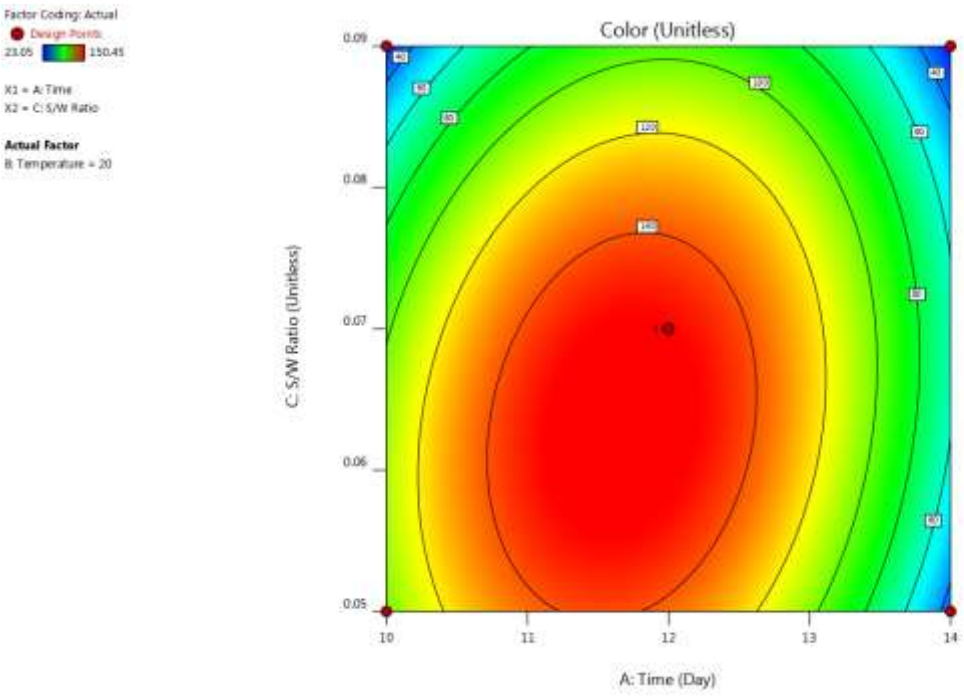

Figure 4.17: Contour plot of interactive effect of antioxidant activity on time and s/w ratio on stability of fresh product

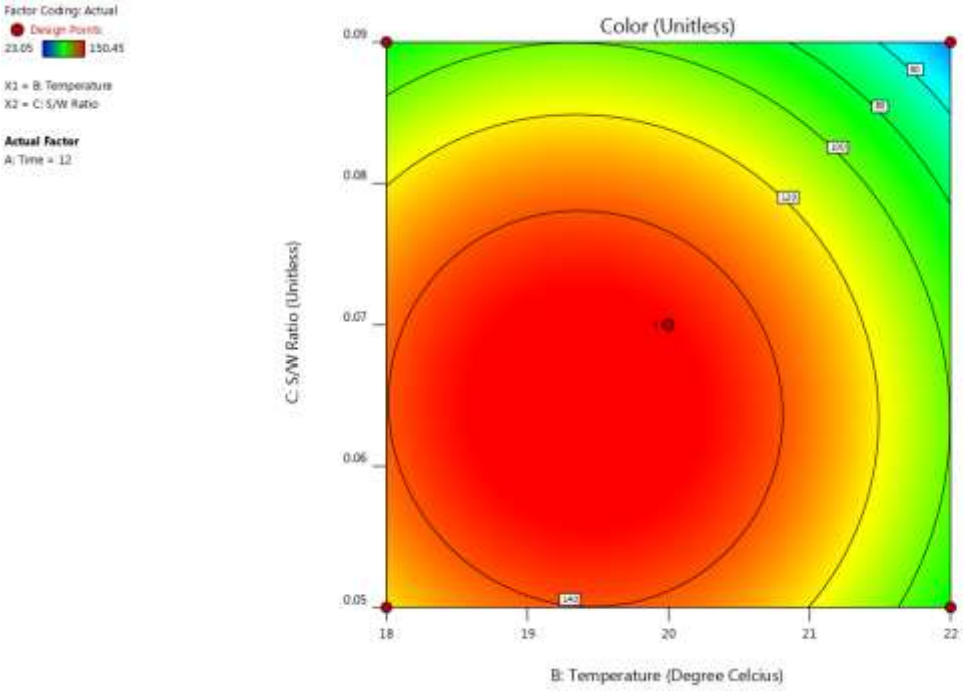

Figure 4. 18: Contour plot of interactive effect of antioxidant activity on temperature and s/w ratio on stability of fresh product

Table 4.6: Optimum condition suggested by Box-Behnken response surface methodology model

Factor name	Optimized level	Total phenol content(TPC)		Antioxidant activity(FRAP)		Color	
		Observed	Predicted	Observed	Predicted	Observed	Predicted
Time	12.15	142.37	140.37	31.38	31.38	150.45	150.45
Temperature	19.42						
s/w ratio	0.089						

4.4.Conclusion:

Red amaranth leaves though rich in bioactive compounds are commercially important leafy vegetables but highly perishable in nature. MAP was applied to store this vegetables in fresh condition without addition of any chemical preservative .It was found that the total phenol, antioxidant activity and color of red amaranth leaves stored under normal atmosphere drastically decreased after 3 days however, samples stored under MA showed four times increase in selflife and acceptability. MA technique is ideal as it delays degradation of leaf pigment. The p value ($p<0.05$) of the proposed regression model was in good agreement with the experimental data.

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Chapter 5

Extraction and Identification of natural color of red amaranth leaves by different solvent

5.1. Introduction:

Amaranth leaves are nutritious leafy vegetables. They grow in the lowland tropics of Asia and Africa. They are of two types- green amaranth and red amaranth. Red amaranth looks wonderful with purple or fully red in color. It is a rich source of calcium and iron, vitamin C and protein [1]. Because of these it acts as a natural antioxidant to fight against infectious diseases like heart disease, cancer, serious eye disease, and muscular degeneration, [2]. Bioactive compounds are present in red amaranth leaves. Bioflavonoid was the most important topic for recent research mainly for their beneficial effects on human health and applications as potential sources of natural food dyes. Natural pigments are used as an eco-friendly alternative to synthetic dyes and also in textile industry. In recent years color plays an important role in the consumer acceptability of food. Colorants are being used in food industry since centuries to enhance or at least restore original appearance of foods. And ensure uniformity as an indicator of food quality. Color is the first characteristic perceived by the senses. Synthetic colorants have always been a question of controversy regarding their safety. Consumers prefer natural colorants than the synthetic ones, as they are increasingly concerned with the safety of synthetic colorants. Therefore, interest in natural colorants has significantly increased because of both legislative action and consumer awareness [3]. The present study deals with some solvent extraction of natural color from red amaranth leaves using aqueous extract, 1% methanolic HCL, 2% methanolic HCL, 2% methanolic citric acid and 1% glacial acetic acid, as well as the identification and quantification of the potent pigment by high-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) and LC-MS.

5.2. Materials:

Red amaranth leaves were purchased from market. HPLC grade acetonitrile, methanol, acetone, acetic acid, 36% pure hydrochloric acid, Millipore, ferric chloride ($FeCl_3$) was purchased from Merck. 2,2-azinobis (3 ethylobenzothiazoline-6 sulphonate) (ABTS), triphenyltriazine(TPTZ), 2,4,6-tris(2-pyridyl)–1,3,5-triazine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (trolox), sodium peroxy disulfate, Folin – Ciocalteu reagent, and gallic acid were purchased from Sigma- Aldrich. Laboratory grade De-ionised water was used. All reagents were of analytical grade.

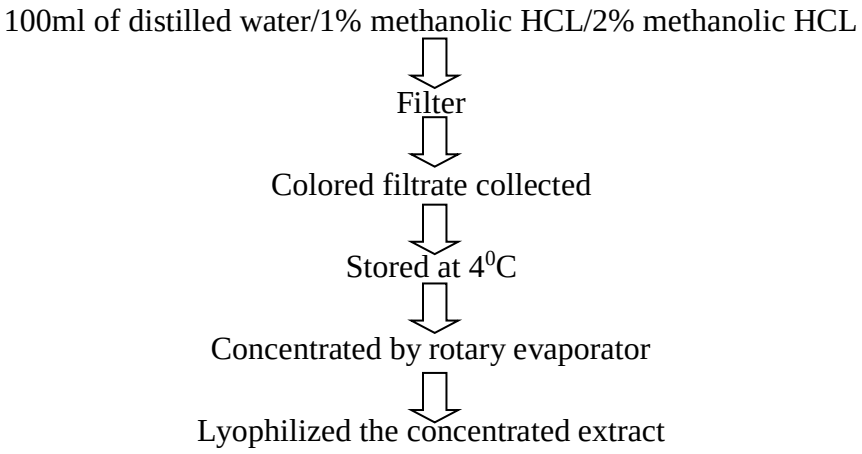
5.3. Methods:

5.3.1 Extraction Process:

Then 5 gm of dried leaves were taken as sample and extraction of color was made with following solvents-

1. Aqueous extract
2. 1% methanolic HCL
3. 2% methanolic HCL
4. 2% methanolic citric acid
5. 1% glacial acidic acid

Flow diagram for (1, 2&3):



Flow diagram (4):

0.01% Ethanolic citric acid

⇩

Green color extracted (300 ml)

⇩

Residue was extracted by 2% methanolic citric acid

⇩

Greenish-red color mixed color collected (600ml)

⇩

100ml red color extracted

⇩

500ml purple color extracted

⇩

Final extract was concentrated by rotary evaporator

⇩

Lyophilized the extracted color

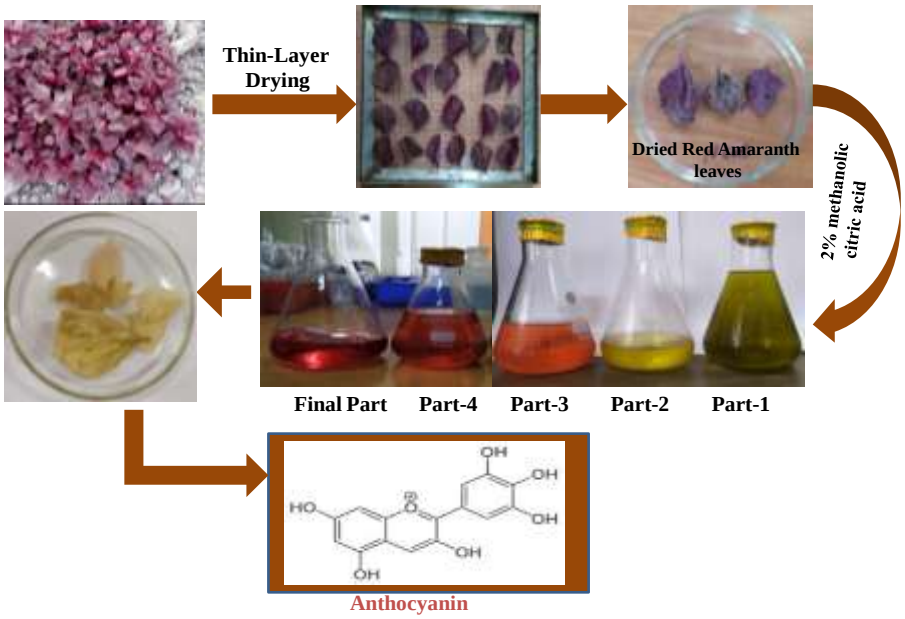


Figure 5.1: Extraction of color pigment from red amaranth leaves by 2% methanolic citric acid

Flow diagram (5):

100ml of 1% glacial acetic acid

⇩

300ml color extracted

⇩

Final extract was concentrated by rotary evaporator

⇩

Lyophilized the extracted color

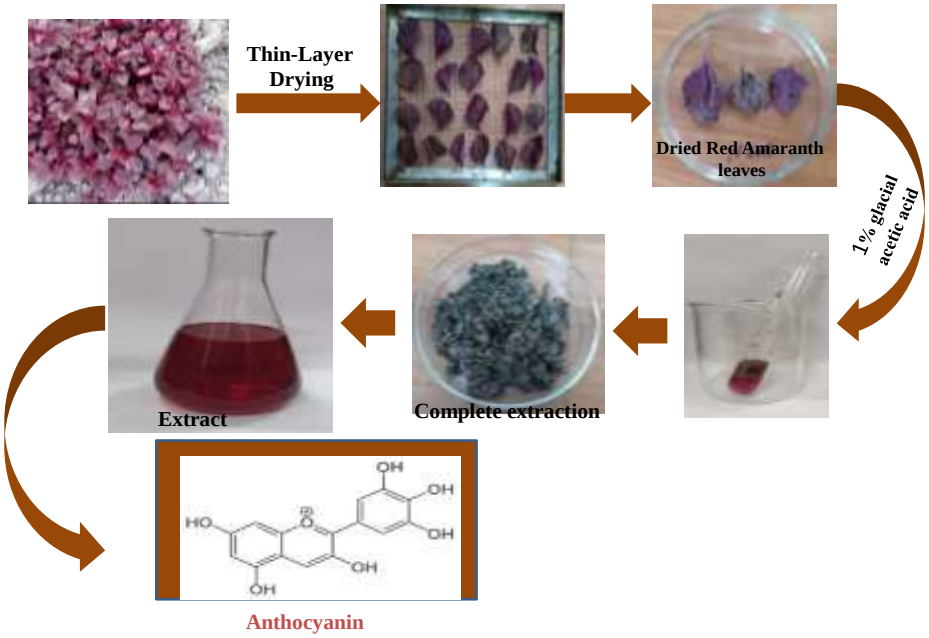


Figure 5.2: Extraction of color pigment from red amaranth leaves by 1% glacial acetic acid

5.3.2 Determination of Moisture Content: 10 g sample of red amaranth leaves were weighed accurately in a dry Petri dish and dried at 105˚C for 3 h. The sample is allowed to cool in desiccators, reweighed and the weight is recorded. The drying- cooling- reweighing procedure is repeated until the weight is constant. The moisture content is measured using the following equation:

$$\text{Moisture content (\%)} = (A\text{-}B/B)*100$$

Where, A is the weight of the moist sample and B is the weight of the dry sample.

5.3.3 Determination of Ash Content: The porcelain crucible was precisely weighed and then the crucible was filled with 3 g of ground sample, and it was weighed. The sample was kept in an electric muffle furnace for six hours at a temperature of 625^0C. Cooling in a desiccator for 15minutes. After that measured the weight of the crucible containing ash. The ash content is calculated using the following equation:

$$\text{Ash content (\%)} = (C-A)/(B-A)*100$$

Where:

A= weight of empty crucible (gm)

B =weight of crucible and sample (gm)

C=weight crucible and ash (gm)

5.3.4 Determination of FRAP activity:

The FRAP assay was determined according to the method of Li et al. (2012)[4]. In this process Ferric (Fe3+) ion was reduced to ferrous (Fe2+) ion. And the intensive blue colored was observed because of the formation of ferrous-tripyridyl-s-triazine (TPTZ) at maximum absorbance 593nm. Absorbance was measured after 8 min and was proportional to the combined ferric reducing/antioxidant power of the antioxidants in the extracts. The percentage of inhibition of DPPH by the leaf extract was calculated according to the following equation:

$$\text{\% of Inhibition} = (1-A_{\text{test sample}} / A_{\text{blank}}) \times 100$$

Where,

A_{blank} = Absorbance of the methanolic blank

And $A_{\text{test sample}}$ =Absorbance of the leaf extracts.

5.3.5 Determination of total phenol content: Described in Chapter 3[4].

5.3.6 Determination of total anthocyanin content: The total anthocyanin pigment content was estimated by the pH differential method [5]. 1 g of the defatted sample was extracted with 1 ml

of acetonitrile containing 4% acetic acid. The absorbance was measured at 510 nm and 700 nm in buffers at different pH level (pH 1.0 and 4.5) using a (U-1800, HITACHI, Tokyo, Japan) spectrophotometer. The total monomeric anthocyanin was calculated by using the following equation:

$$A = [(A_{510}-A_{700}) \times pH1.0 - (A_{510}-A_{700}) \times pH4.5]$$

Molar extinction coefficient of cyanidine- 3-glucoside was 26900 molar in L. $mol^{-1}.cm^{-1}$. Results were expressed as milligrams of cyanidine-3-glucoside equivalent (CGE) 100 g_1 DW.

5.3.7 Identification of red amaranth leaves aqueous extract by HPTLC methods:

The chromatographic strategy permitted noticing varieties in the substance of specifically anthocyanins, which is incredible by the photometric approach. To improve the separations durability and reduce analysis time, HPTLC (High performance thin layer chromatography) can replace HPLC. However, applying chromatographic analysis to HPTLC data is difficult, and effective data preparation requires a specific approach [6,7]. HPTLC is traditionally used for authenticity studies of medicinal plants based on the pattern of their secondary metabolites. For all analyses, HPTLC plates (100 × 100 mm, 200 μm silica gel 60 F254, glass plates ; Merck, Darmstadt, Germany) were used. Samples thawed and filtrated through a 0.45 μm PTFE syringe filter (VWR International, Darmstadt, Germany) into amber vials and anthocyanin standards, i.e. kuromanin (cyanidin 3-*O*-glucoside) chloride (Extrasynthese, Genay, France), was applied with an Automatic TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland), using the following settings for 18 sample tracks per plate: band length 8.0 mm, track distance 10.0 mm, dosage speed 50 nL/s , first application position: 15 mm from the left edge (x-axis) distance 13.4mm, 8 mm from the bottom edge (y-axis); 14 μl of each sample and standard were applied. Development was carried out in an automated developing chamber (ADC2, CAMAG, Muttenz, Switzerland) with the following settings: migration distance 60 mm, 20 min saturation, 10 min activation using molecular sieve for a relative humidity of 0–5%; 5 min drying time. The same solvent mixture was used for both plate development (10 mL) and saturation (25 mL): Acetonitrile: water: Trifluroacetic acid (20:80:2, v/v). Scanning densitometry was performed

with a TLC Scanner 3 (CAMAG, Muttenz, Switzerland) both directly after sample application and after development at 535 nm using the following settings: scanning speed: 20 mm s^{-1}; data resolution: 100 μm per step; slit: 5 × 0.2 mm, micro. All instruments were controlled with VisionCats 2.0 software (CAMAG, Muttenz, Switzerland). Peak heights (intensities) and peak areas were evaluated with win CATS 1.4.9 software (CAMAG, Muttenz, Switzerland). Peaks with intensities less than 2 AU were ignored and only peaks with a retention factor (*Rf*) between 0.2 and 0.7 were considered. In total, ten target zones at *Rf* 0.2, *Rf* 0.28, *Rf* 0.34, *Rf* 0.37, *Rf* 0.45, *Rf* 0.49, *Rf* 0.52, *Rf* 0.59, *Rf* 0.65 and *Rf* 0.70 were obtained. Shifts in *Rf* between plates were small and easily corrected by the anthocyanin standards and four check samples which were included in each plate. Peak heights and areas were used for statistical analysis. TAC determination with à côté calibration was conducted according to Oberlerchner et al., [8]. In brief, kuromanin chloride standards were applied to each plate outside the area required for chromatography (70 mm, y-axis), while the samples were applied at their usual position near the bottom edge (8 mm, y-axis). The plates were scanned at 535 nm directly after application of the standards and then again after sample application. Then, chromatography was performed as described above. The peak areas of the standards acquired in the first scan were used for calibration, establishing a linear relationship of the square root of the peak area and the decimal logarithm of the kuromanin concentration. This calibration was then used to convert the areas of the application spots, which had been determined in the second scan, into kuromanin-equivalents (kur-eq) per gram bran.

5.3.8 Identification of red amaranth aqueous extract by HPLC methods:

For the past 50 years, HPLC analysis has been the method of choice for anthocyanin identification and quantification [9,10,11&12]. This method became more applicable with the development of reverse phase columns that can separate anthocyanins and photodiode array detection (PDA). C18 columns, a detection wavelength of 520 mm, acetonitrile, and water solvent systems with pH-adjusted phosphoric acid, formic acid, or trifluoroacetic acid are the standard HPLC settings. In qualitative and quantitative research of anthocyanins and anthocyanidins, HPLC techniques have gained popularity due to their simplicity and sensitivity.

For removing the sugar and acid from the extracts 1% trifluoroacetic acid (TFA) in methanol was added to it and then it was pass through a supelclean LC-18 cartridge [13]. A high performance liquid chromatography (HPLC) (Alliance 2,695 HPLC system; Waters Corporation,

Massachusetts, MA) equipped with a binary pump, a dual λ absorbance UV detector 2,487, an inline degasser, and the Empower 2 software. The separations were performed on using Symmetry C-18 reversed-phase column (250 mm × 4.6 mm length, 5 µm particle size) at 30 ^{0} C. Acidified water with 0.1% TFA (v/v) was used as mobile phase A and HPLC grade acetonitrile with 0.035% formic acid (v/v) was used as phase B. The injection volume was 20 µl. The following gradients were used: 10% - 11% of B in 12 min, 11% - 12% in 8 min, 12% - 13% in 5 min, 13% - 18% in 10 min, and 18% of B maintained for 25 min. The anthocyanins were analyzed with a visible detector at 520 nm. Before injecting it, the sample was filtered through a 0.45 µm acrodisc (Millipore, Bedford, MA). The flow rate was kept at 1 ml /min. For the identification of anthocyanin cyaniding-3-glucoside was used as a standard. The amount of phenolic compound was expressed as milligram per gram of the extract.

5.3.8 Qualitative and quantitavive analysis of aqueous red amaranth leaves by LC-MS:

Analyses were performed by using a Waters Xevo –G2 XS Q-TOF mass C18 column (100 × 2.1 mm i.d.; particle size 5 µm) and as mobile phase a gradient of 0.1% formic acid as eluent A and acetonitrile (LC-MS grade, J. T Becker) as eluent B. The flow rate was 0.250 ml /min and the amount injected was 10 µl. Here, the column back pressure was used as1490 bar and the temperature was set at 25 °C. An electro-spray ionization source (source block temperature 130 °C, desolvation temperature 300 °C, capillary voltage 3 kV, cone voltage 35 V) was used when the desolvation and cone gas flow rate were 650 and 50 L/h respectively. The flow generated by chromatographic separation was directly injected into the electro-spray ion source for mass detection. Positive ion as well as negative ion mode was used for mass detection.

5.4. Results and Discussion:

5.4.1 Composition of fresh red amaranth leaves:

From Table 5. 1 the moisture content of red amaranth leaves is 89.39%. The ash content of the sample is 1.25 per gram of sample, which indicated the amount of micronutrient composition in red amaranth leave. The total phenol content of red amaranth leaves is161.29µgFAE/g dw. Antioxidant content of red amaranth leaves is 35.13 (% inhibition). Results indicated that the extracted red amaranth leaves contained about 161.90 mg/100 g on fresh weight of anthocyanins

pigments; the results declared that red amaranth leaves contained high concentration of total anthocyanins. These results are in agreement with that obtained by M.T.M. Assous *, M.M. Abdel-Hady, Ghada M. Medany (2014).

Table 5.1: Composition of fresh red amaranth leaves (Dry basis)

Moisture Content (%)	**Ash Content (%)**	**Total Phenol Content (µg FAE/g dw)**	**Antioxidant Activity (% of inhibition)**	**Total Anthocyanin Content (mg/100g of fresh sample)**
89.39	1.25	161.29	35.13	161.9

5.4.2 Identification of red amaranth leaves aqueous extract by HPTLC methods:

A high-performance thin-layer chromatographic (HPTLC) method was developed for the Identification and quantification of anthocyanin, with respect to the standard cyanidin-3-O-glucoside chloride from the Red Amaranth leaves. The densitometric reflection scanning profiles of HPTLC Chromatograms of 2% methanolic citric acid, 1% glacial acetic acid and the standard at 530nm on silica gel are shown in figure.5.1, figure 5.2 & figure 5.3. Good resolution & spread of R_f values are observed for the liquid phase and this is suitable for quantitative analysis for anthocyanin.

Table 5.2: Substance AS R_F (0.657=/- 0.288)

Track	**R_F**	**X(mm)**	**Y(mm)**
1	0.432	15.0	39.1
2	0.825	28.4	67.4
3	0.943	41.8	75.9
4	0.408	55.2	37.4
5	0.411	68.6	37.6
6	0.822	82.0	67.2

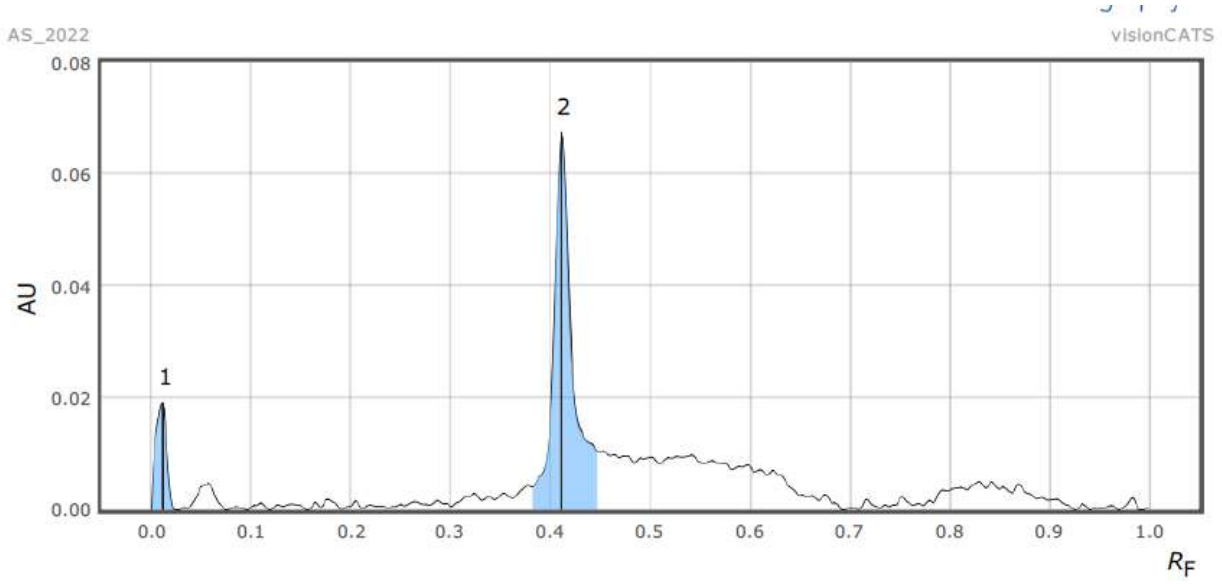


Figure 5.3: HPTLC densitogram of red amaranth leaves extracted by 2% methanolic citric acid at 520 nm

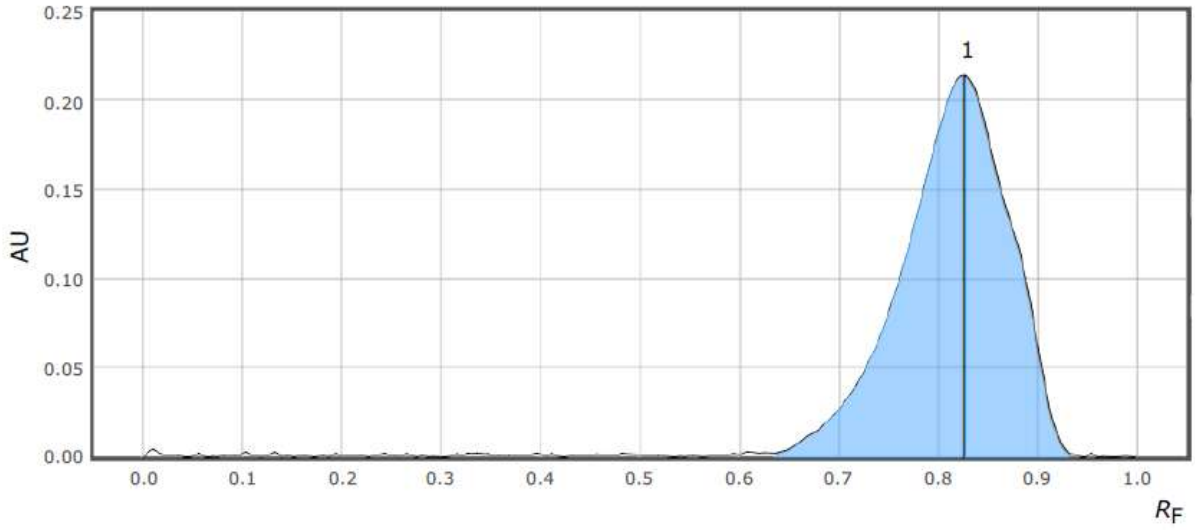


Figure 5.4: HPTLC densitogram of red amaranth leaves extracted by1% glacial acetic acid at 520 nm

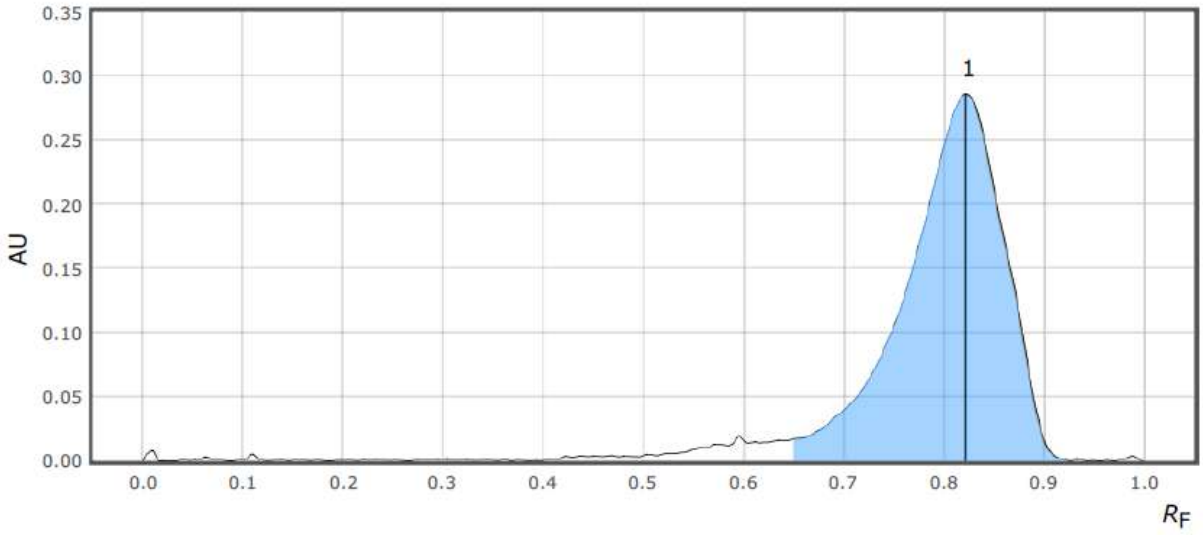


Figure 5.5: HPTLC densitogram of standard solution at 520 nm

Cyanidin-3-glucoside chloride was used as a Substance for the quantitation of anthocyanin in the samples where and absorbance was measure at 520nm.

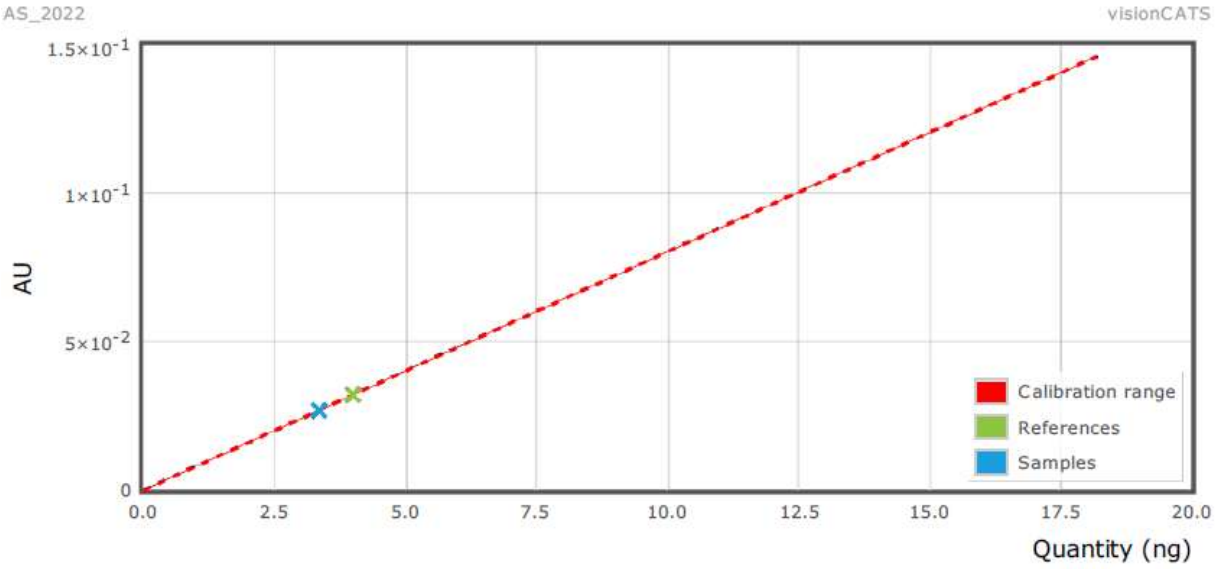


Figure 5.6: Calibration curve for red amaranth leaves at 520nm

This is a regression linear equation $y=8.032\times10^{-6}$ x. Polynomial calibration of cyaniding-3-glucoside chloride via peak hight by absorption at 520 nm gives 1.677µg anthocyanin present in 1ml of 1%glacial acetic acid extract.

5.4.2 Identification of different solvent extraction of red amaranth leaves by HPLC methods:

HPLC methods are applied for separation and purification of bioactive compounds in plant samples. It also helps to identify the accurate structure of bioactive compound from natural sources [14]. This analytical technique is used for the qualitative or quantitative analysis of non-volatile compounds like phenolics, terpenoids, and alkaloids [15].These highly capable methods accelerate the analytical separation with higher sample loading capacity [16,17]. Estimation of qualitative analysis of the analyzed sample depends on the stability of retention time of the referred standard. When the standards are injected at different dilution levels quantitative estimation is performed by standard curve obtained [18]. Five major peaks have been identified by HPLC for the aqueous red amaranth leaves extracts. Here, we are using cyanidin-3- glucoside as a standard. Cyanidin-3-glucoside gave a maximum absorption at 518nm. Peonidin, delphilidin, petunidin and malvidin had similar spectra with maximum absorption at wavelength 524nm, 530nm, 532nm, and 534nm respectively. Anthocyanins pigments extracted from red amaranth leaves by aquous extract, 1% methanolic HCL, 2% methanolic HCL,2% methanolic citric acid and 1% glacial acetic acid were separated and identified by HPLC are shown in fig.1,2,3,4,5 respectively.

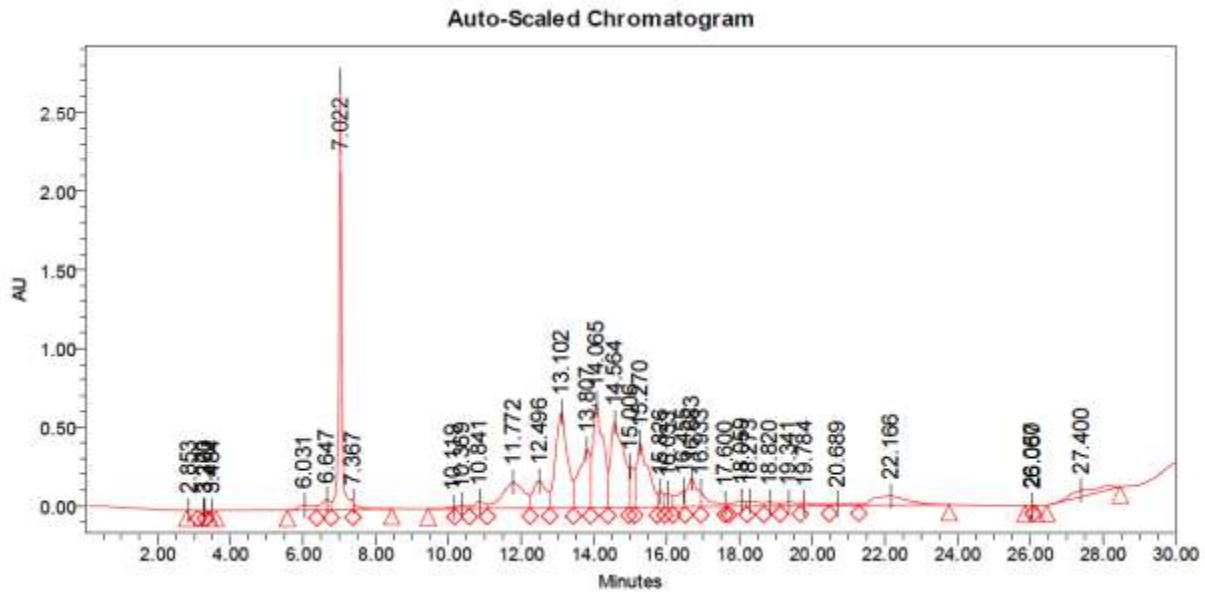


Figure 5.7: HPLC chromatograph of aqueous extract of red amaranth leaves at 254nm

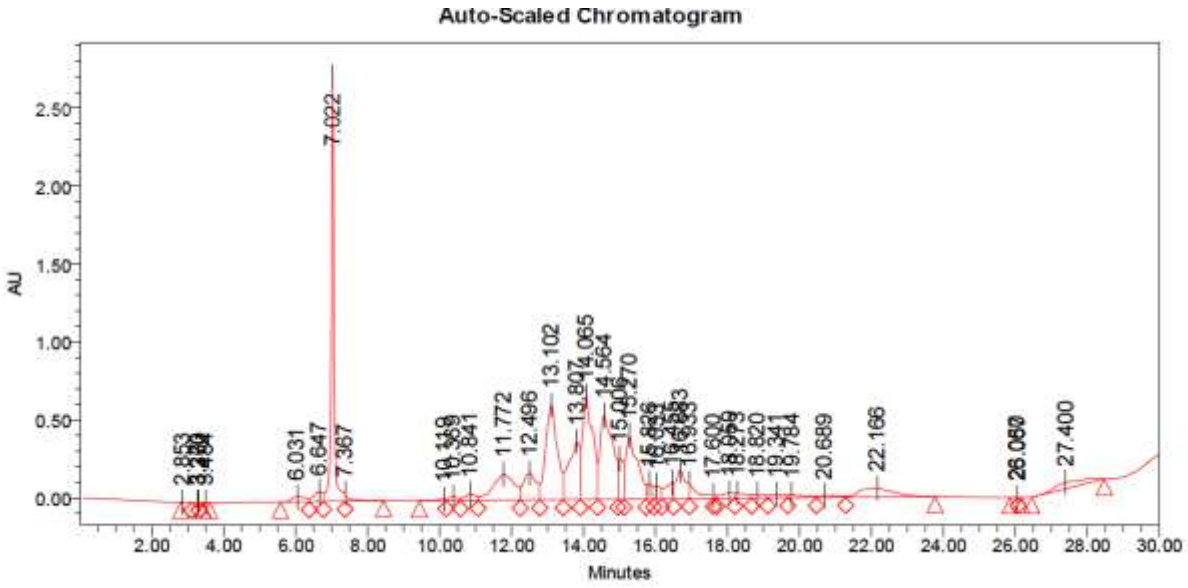


Figure 5.8: HPLC chromatograph of 1%methanolic HCL extract of red amaranth leaves at 254nm

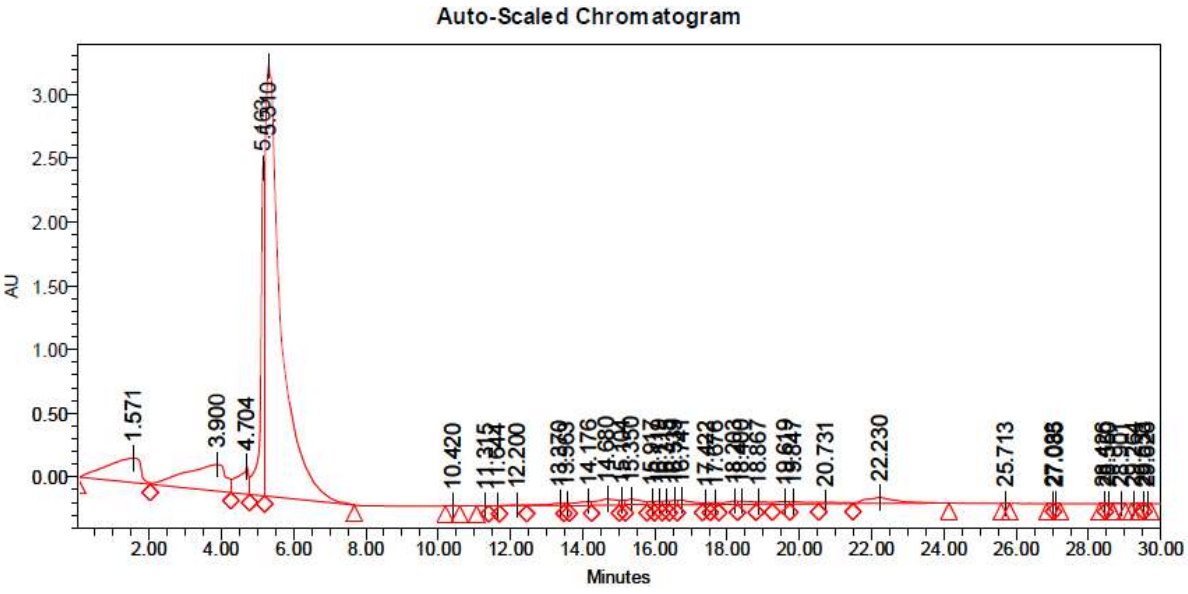


Figure 5.9: HPLC chromatograph of 2%methanolic HCL extract of red amaranth leaves extract at 254nm

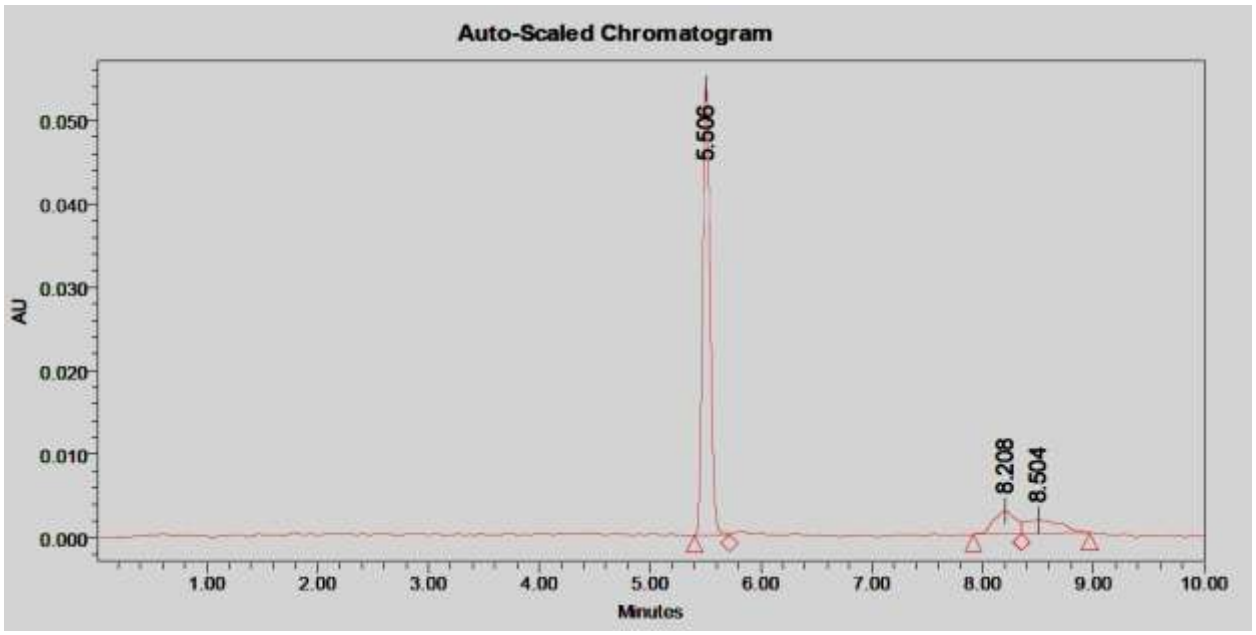


Figure 5.10: HPLC chromatograph of 2% methanolic citric acid extract of red amaranth leaves at 520nm

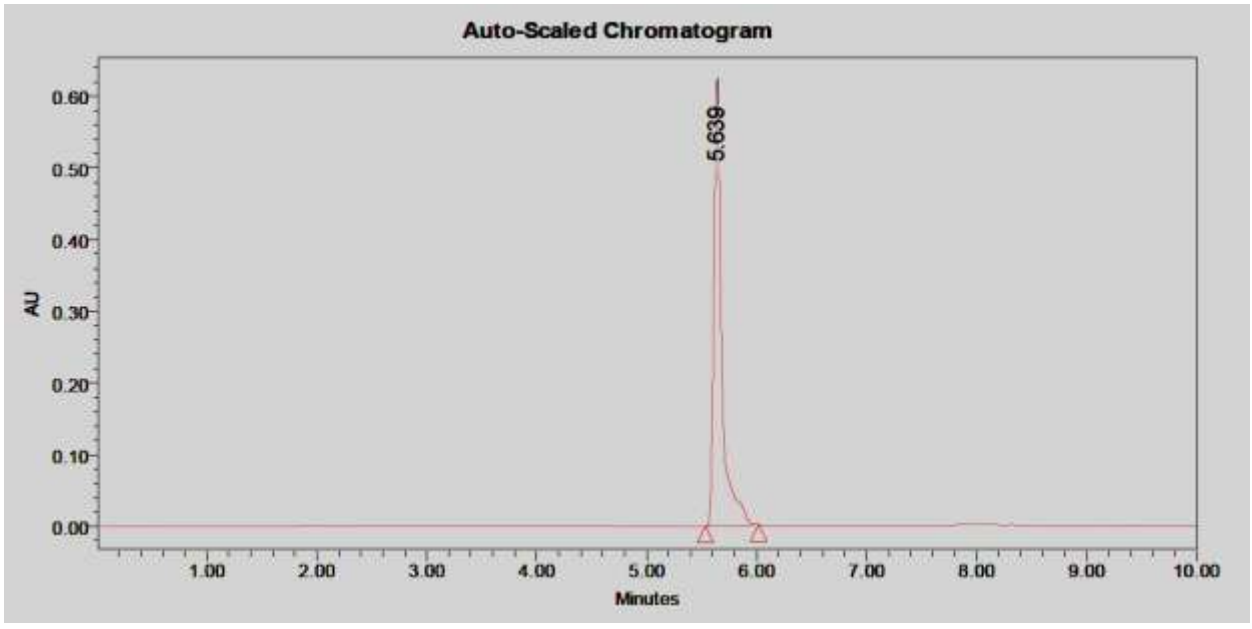


Figure 5.11: HPLC chromatograph of 1% glacial acetic acid extract of red amaranth leaves at 520nm

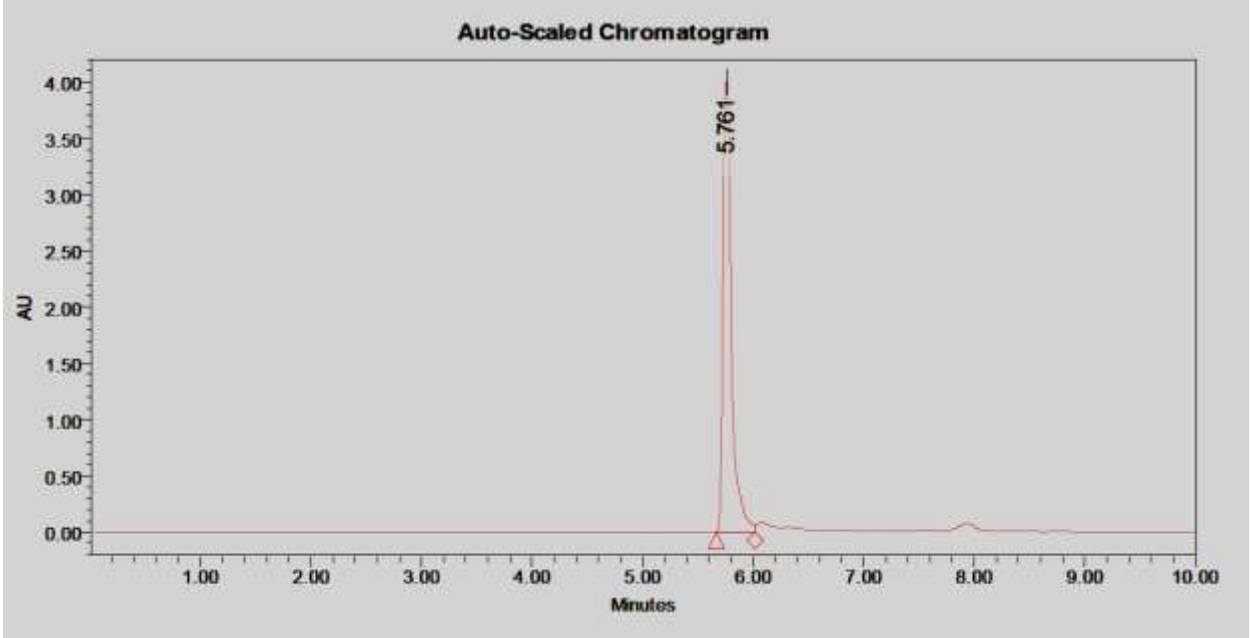


Figure 5.12 : HPLC chromatograph of Standard curve of anthocyanin at 520 nm

Figure 5.7, Fig 5.8, Fig 5.9, Fig 5.10, Fig 5.11 & Fig 5.12 no figure represents HPLC chromatogram of red amaranth leaves aqueous extract, 1% methanolic HCL and 2% methanolic HCL , 2% methanolic citric acid , 1% glacial acetic acid and the standard curve of anthocyanin (cyaniding-3-glucoside chloride): Aspertic acid (6.7min), gallic acid (7.0 min), protocatechuic acid (7.2 min),), vanilic acid (8.4 min), anthocyanin(11.03), catechin (12.5) min, rutin (12.8 min), trans-cinnaminicacid (13.0), ferulic acid (13.1 min), quercetin (13.9min) and kampferol(14.5)

Fig. 5.5 represents the HPLC of aqueous extract of red amaranth leaves. Five phenolic compounds from the aqueous red amaranth leaf extracts separated and successfully identified by using a HPLC method .It shows five different peak at different retention time (6.661, 7.743, 11.086, 12.814 and 14. 399). Aqueous extract has aspertic acid, proto-catechuic acid, anthocyanin, rutin and kampferol. From Figure 5.12 the standard curve of cyaniding-3-glucoside we have to see the retention time of anthocyanin is 11.038. Therefore, from Figure 5.7, we have to conclude that anthocyanin was present in the aqueous extract of red amaranth leaves. From Figure 5.8 &Figure 5.9 many phenolic compounds are separated and successfully identified by using HPLC methods. It shows many different peak at different retention time. In comparison to Figure 5.12 we conclude that anthocyanin is present in these two extracts. But his two extraction

methods are not suitable for the extraction of anthocyanin from red amaranth leaves due to the presence of many phenolic compounds.

Figure 5.10, & Figure 5.11 represents HPLC chromatogram of red amaranth leaves extracted from 2% methanolic citric acid, 1% glacial acetic acid. Three phenolic compounds from 2% methanolic citric acid extracts separated and successfully identified by using a HPLC method (Fig. 5.10).It shows three different peak at different retention time (5.506, 8.208 & 8.504).This Citric acid extract have anthocyanin, rutein & proto-catechuic acid. From the standard curve of cyaniding-3-glucoside we have to see the retention time of anthocyanin is 5.761. Therefore, from Fig.5.10, we have to conclude that anthocyanin was present in the 2% methanolic citric acid extract.

Only one phenolic compound from 1% glacial acetic acid extracts separated and successfully identified by using a HPLC method (Fig. 5.11).Fig-5.11 shows one peak at the retention time 5.639. This glacial acetic acid extract have content only anthocyanin. From the standard curve of cyaniding-3-glucoside we have to see the retention time of anthocyanin is 5.761. Therefore, from Fig.5.11, we have to conclude that anthocyanin was present in the 1% glacial acetic acid extract.

5.4.3 LC-MS of red amaranth leaves aqueous extract:

Aqueous extract was more effective than the other two extraction methods. So, the aqueous extract was used for characterization of anthocyanin by LC-MS. A mass spectrometer combined with a liquid chromatography can detect masses characteristic of a compound or of a class of compounds. The system can selectively detect compounds of interest in a complex matrix, thus making it easy to find and identify suspected impurities at trace levels. Presently, LC-MS is the most popular identification method for bioactive compounds from natural sources. LC-MS provides the exact or predicted molecular weight of the extract sample. It also gives the structural identification by calculating the accurate mass to charge (m/z) ratio of the compounds [C.D. Stalikas et al., 2010]. Polyphenols are the most common bioactive compounds in plant sample.LC-MS is the most efficient method for separation of flavonoids from natural sources. Flavonoids are detected depending on the chemical composition. UV absorption spectrum and the retention time of the reference standard indicated the chemical composition of the compounds. If the retention times of two components are nearly same then on the basis of multiple wavelength detection systems absorption spectra is separated. Flavonoids have O-

glycoside and C-glycoside as a result the substitution pattern of methoxy and hydroxyl group are in small wavelength range of both bands. In flavonoids analysis ESI, APCI are used as a ionization source. Mass Spectrometry imparts structural information of phenolic compounds by identifying the distribution pattern of the components [19]. In several cases due to the presence of impurity in the sample the analyzed compound may not reached the accurate corresponding fragment ion.

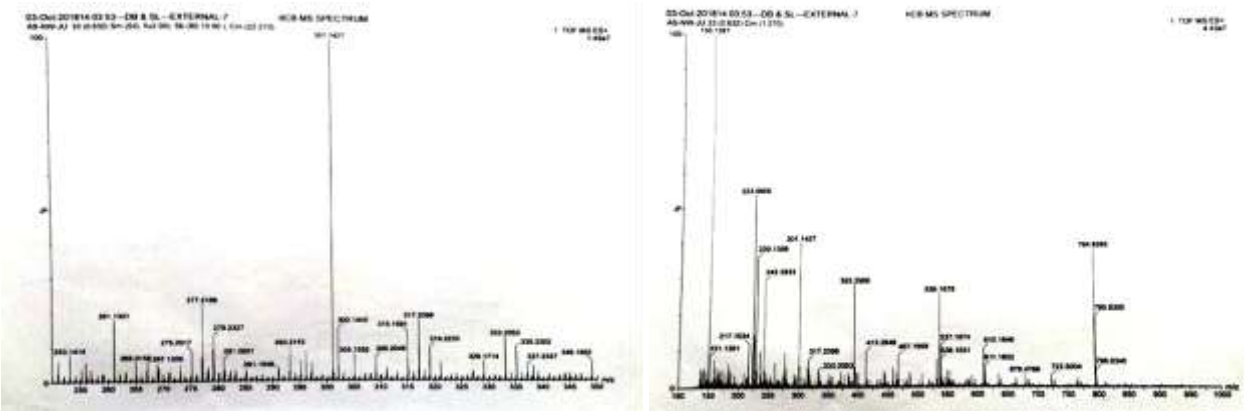


Figure 5.13: Mass spectrum of aqueous red amaranth leaf extract.

The volatile compounds are usually present in plant polyphenol as acylated with aliphatic and aromatic acids to the glycosidic part. So it is very difficult to identify the structure of acylated flavonoid glycosides. Figure 5.13 shows the LC-MS of aqueous extract of red amaranth leaves .The mass spectrum of this unknown compound contained some fragments at m/z 287, (cyanidin), m/z 303, (delphinidin),m/z 317 (petunidin), m/z331 (malvidin), m/z 435 (cyaniding-3-arabinoside), m/z 449 (cyaniding-3-galactoside), m/z 465(delphinidin -3-glucoside and delphinidin -3-galactoside),m/z 493(malvidin-3-glucoside). This result shows that anthocyanin was present in the aqueous extract of red amaranth leaves.

5.5 Conclusion:

Red amaranth leaf contains a higher amount of phenolics and anthocyanins , and it also possesses antioxidant properties. Further scientific analysis of red amaranth leaf will be aided by the composition of the main phenolic and anthocyanin constituents, total phenol content, total antioxidant content, total anthocyanin content, and colour characteristics. Anthocyanins may

have been the main antioxidants in red amaranth leaves based on the strong positive correlations between bioactive phytochemicals (TPC and TANC) and antioxidant activity (FRAP), suggest that anthocyanin, rutin, catechin, quercetin and other phenolic compounds, as well as amaranthines were the main antioxidants in red amaranth. So we have to conclude that anthocyanin pigment is present in the aqueous extract of red amaranth leaf. From HPTLC, 1% glacial acetic acid extract a fourfold higher concentration of the total anthocyanin was found compared with the 2% methanolic citric acid extract. HPLC shows a similar profile.LC-MS further clarify that anthocyanin is present in red amaranth leaves.

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Chapter 6

Application of natural color extracted from red amaranth leaves

6.1 Introduction:

Color is thought to be the major sensory element that has played a significant part throughout history in determining whether or not food is accepted in order to improve its real quality and appearance. Plant pigments, the ideal natural source of colour, have a great deal of promise to replace many synthetic colouring agents. Plant pigments have a lot to offer the food sector, and those perspectives are positive. There has been an increase in the use of natural colourants. Renowned for their therapeutic and medicinal benefits across the world .Consequences in addition to their high toxicity [1,2].Anthocyanins are polyphenols with known antioxidant activity which are responsible for the red, blue, purple colors of plant assets like organic products, blossoms, and leaves. Anthocyanins are mostly found in berries, such blackberries, grapes, and blueberries, as well as some vegetables, like egg-plants (aubergine) and avocados. Other foods that are important parts of the human diet include oranges, elderberries, olives, red onions, figs, sweet potatoes, mangoes, purple corn, pomegranates, and red amaranth. Anthocyanins may be used in the food, drug, and cosmetic sectors. In the food sector, the vegetable pigments carotenoids and anthocyanins are most frequently used. Commercially produced anthocyanin-based colourants for used in food are created from horticulture crops and processing waste. In the literature, there is a wealth of information on the chemistry of anthocyanins, including conventional methods, organized techniques for classifying anthocyanins, calculating their concentrations, and analysing their colour characteristics. Recent research has demonstrated that anthocyanins exhibit a variety of biological actions. They can be employed as antioxidants, which are thought to have several positive health effects. In addition, it contains anti-inflammatory, antibacterial, and anti-carcinogenic properties. Furthermore, they show different impacts on veins, platelets, diabetes, joint pain, and lipoproteins ready to decrease the gamble of coronary heart sicknesses. In the current work, utilization of anthocyanin extracted from red amaranth leaves in food industry.

Currently, additives or supplements made from pigments of various types and forms are utilized in the food sector, cosmetics, pharmaceuticals, animal feed, and other uses [3]. Nevertheless, due to the issues of the synthetic colors that cause harmfulness and cancer-causing nature in the human body, their use has gradually declined. As a result, there is growing interest in natural pigments that can replace synthetic ones and avoid their negative side effects [4,5]. Recently, in

response to this trend, there has been a progressive expansion of the use of natural pigments as well as the addition of natural materials in natural dyeing, healthy functional foods, and cosmetic goods for human health and safety [6,7,8,9].

India is the largest producer of milk and contributes 23% of the World's total milk production. Milk production was 209.9 million tonnes (provisional) in the year 2020-21. According to data from Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), milk production was 209.96 million tons (MT) during 2020-21 against 198.44 MT in 2019-20. India accounted for 21 percent of the global output. Among 100% of total milk production, 46% milk is consumed as a liquid form while remaining 54% used for the preparation of milk products, in which 7% is used for the preparation of converted into heat and acid coagulated indigenous milk products among which Kalakand is one of the products [10]. Kalakand is a popular Indian sweet prepared from solidified, sweetened milk, it was invented in 1947 by Baba Thakur Das in Alwar, Rajasthan, India. Fruits/vegetable juices or pulp has been used for the fortification process to improve the taste and acceptability of kalakand [11]. It is popular in north-east India including Jharkhand, Orissa and Bengal states. It is originated in Braj area of western Uttar Pradesh [12]. Kalakand is one of the major heat desiccated indigenous popular traditional Indian sub-continent milk product, which is gaining more popularity in modern societies of the developing countries. It contains milk solids in a fourfold concentration, its food and nutritive value is very high. Milk based sweets are an integral part of the dietary system of Indian sub-continent. These sweets are specially served on various occasions such as weddings, festivals and celebrations. Traditional milk products in India have great commercial importance as they account for over 90 % of all the milk products consumed in the country [13]. Being the largest producer in the world, India has produced about 140 million tons of milk annually in the year 2013 (Govt. of India Report 2013). It is estimated that about 50-55 % of this milk produced is converted into traditional dairy products like heat desiccated milk products viz., Khoa, Basundi, Lalpeda, Rabri, Kalakand etc., coagulated milk products viz., Dahi, Shrikhand, Paneer, Chhana and Chhana based products and heat clarified products viz., butter oil, Ghee etc., which have a strong social and cultural heritage in the Indian society. Kalakand is prepared by blending sugar with 'danedar' Khoa and heated for giving it a pleasant caramel flavor and granular texture. The color of Kalakand varies from off white to light caramel [13].

Table 6.1: Nutritional value of red amaranth leaves:

Nutritional parameter	**Nutritional Value(100gm)**
Carbohydrate	5.0 gm
Protein	5.3 gm
Fat	0.1gm
Vitamin C	43 gm
Total phenol	162.36±1.64µgFAE/g dw
Antioxidant	37.23±3.91(%inhibition)

6.2 Material and method:

6.2.1Preparation of Kalakand:

Kalakand was prepared by the procedure suggested by Aneja et al. (2002) with slight modifications[13]. Standardized (manufactured by Amul Slim'n' Trim pasteurized homogenized double tonned milk with fat 1.5% and SNF 9.0 %) milk was taken in a jacketed vessel and heated to simmering temperature (85–90 °C) with continuous stirring by a wooden ladle in a circular motion with occasional scraping of the heating surface. After 20–25 min of boiling, 0.02% of citric acid (in the form of solution) was added to milk to facilitate granulation with vigorous stirring obtain a good quality product. The intensity of heating was reduced when semi-solid state was reached. To this preparation, 6 % sugar (by weight of milk) was added, while continuously stirring the product. The finished product was transferred to tray previously greased with butter for cooling and setting at room temperature. Two sets of kalakand were prepared, one with anthocyanin as antioxidant additive in concentration (chhana:color=50gm:30mg) and the other without anthocyanin extract and they were denoted as Test sample(T) and Control sample (C) respectively. The process diagram along with the mass balance for manufacturing Kalakand is shown in Fig.6.1.

6.2.2Flow diagram of preparation of kalakand:

Standard milk (double tonned milk with fat 1.5% and SNF 9.0 %)

⇩

Heating (85–90 °C), 20-25min

⇩

Addition of 0.02% of citric acid solution

⇩

Desiccation upto 30% moisture

⇩

Addition of 6% sugar (w/w)

⇩

Mixing

⇩

Addition of color (chhana: color=50gm: 30mg)

⇩

Moulding and settiing of Kalakand in a tray

⇩

Cutting

⇩

Stored at 5°C

Figure of colored kalakand and Normal kalakand in below-

Figure 6.1: Normal kalakand and colored kalakand

Kalakand samples were subjected to proximate analysis and texture analysis and sensory evaluation .The chemical characteristics such as titrable acidity (1), fat content (2), protein (3), lactose (3) sucrose" ash total solids" and moisture content (gravimetrically) were analyzed as per standard methods. Kalakand samples after preparation were stored in refrigerator at 5±1˚C for a period of 15 days. Organoleptic and chemical changes were studied during this storage period.

6.2.3 Proximate analysis of kalakand:

Analysis of caloric value, total carbohydrate, acidity, fat content, protein content, lactose and sucrose, total soluble solid content and moisture content of the kalakand samples were determined. The analytical methods were described in Rangana (1986).

6.2.3.1Determination of total phenol and antioxidant activity:

Total phenol content and antioxidant activity (TPC and FRAP assay) of kalakand were done by the method mentioned in chapter1.

6.2.3.2Organoleptic evaluation:

The sensory analysis of samples was carried out at 25 °C by a trained panel of 9 judges drawn from the research scholar and students of the Department of Food technology and biochemical engineering, Jadavpur University, India. The judges were asked to score for the sensory attributes viz. color and appearance, flavor, body and texture, sweetness and overall acceptability on a 9-point Hedonic scale 9 as excellent and 2 as unacceptable product. Moisture and nutritional component analysis .The moisture content and the nutritional composition in Kalakand samples were analyzed using the AOAC (2000) method.

6.2.4 Texture profile analysis (TPA):

TPA on samples was performed by using the Texture Analyser TA.XT plus, Exponent Lite (Stable Micro Systems, Surrey, USA) to characterize the hardness, adhesiveness, springiness, cohesiveness and gumminess of Kalakand. The samples of Kalakand were cut into 1.5 cm3 size pieces and their temperature maintained at 25 °C during the textural analysis. The samples were subjected to mono axial compression of 5 mm height. The force distance curve was obtained for a two bite compression cycle with the test speed of 5 mm/s and trigger force of 5 g.

6.3Results and discussion:

Colored kalakand is attractive due to the color of anthocyanin. This anthocyanin is used as a natural color as well as natural antioxidant.

6.3.1Average data for different parameters of control and experimental Kalakand:

6.3.1.1Physico-chemical analysis Moisture Percentage

There was no significant difference in moisture content. Moisture content of control and colored kalakand 23.14% was found.

6.3.1.2 Fat Percentage

There was no significant difference in fat were observed in different treatment combination. Fat content of control sample was 21.32% and test sample was found 22.17%. The difference in fat was occur due to the fat content of red amaranth leaves.

6.3.1.3 Ash Percentage

There was no significant difference in ash were observed in control and colored kalakand. Ash was found of 2.03%

6.3.1.4 Protein Percentage

There was significant difference in protein were observed in control and colored kalakand(Test sample). Maximum protein of 18.37%was found in the test sample and the protein content of control sample was 15.35%. The difference in protein was occur due to the high protein content of red amaranth leaves.

6.3.1.5Total phenol content:

There was significant difference in total phenol content .Adding anthocyanin as a natural color extracted from red amaranth leaves it's enhance the nutritional quality of color kalakand. Total phenol content was observed 162.36±1.64µg FAE/gdw

6.3.1.6Antioxidant activity:

There was significant difference in antioxidant activity of control and test sample due to the high antioxidant content of red amaranth leaves ((37.23±3.91) % of inhibition).

6.3.2Texture analyser:

Texture analysis parameters namely hardness, cohesiveness, adhesiveness, springiness, gumminess, chewiness was measured (Table. 6.2). Texture profile measurement was carried out using double bite compression test, since, force and deformation are the two fundamental parameters for texture characterization. The peak force during the first compression cycle is defined as hardness or firmness; the ratio of the positive force area during the second compression cycle to that during the second compression cycle was expressed by the property of cohesiveness.

Table 6.2: Texture profile analysis of kalakand by texture analyser

Sample	Hardness	Cohesiveness	Adhesiveness	Springiness	Gumminess	Chewiness
Kalakand	81.823	0.245	-18.379	0.995	20.049	19.950

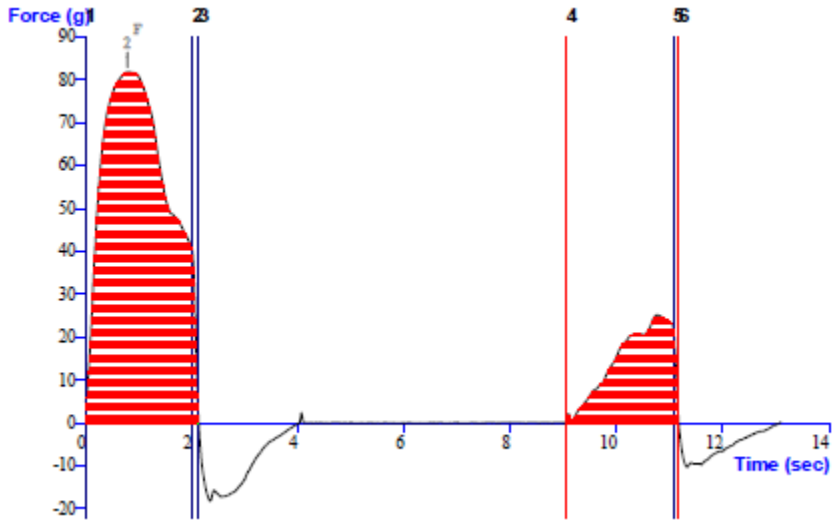

Figure 6.1: Texture profile of kalakand by texture analyser

6.3.3 Organoleptic evaluation: Normal (control) kalakand and colored kalakand were analyzed using 9 point scale for various quality attributes and results are summarized in table 6.3. It is found that the colored kalakand is more acceptable in terms of natural color.

Table.6.3: Sensory analysis of normal and colored kalakand

Sample	Color	Taste	Texture	Flavor	Mouthfeel	Overall acceptability
Control sample	8.4	8.5	8.5	8.6	8.5	8.5
Colored kalakand (2% methanolic citric acid)	8.5	8.5	8.6	8.6	8.5	8.54
Colored kalakand (1% glacial acetic acid)	8.8	8.5	8.6	8.6	8.5	8.6

6.4 Conclussion:

Red amaranth leaf contains a higher amount of phenolics and anthocyanins , and it also possesses antioxidant properties. Further scientific analysis of red amaranth leaf will be aided by the composition of the main phenolic and anthocyanin constituents, total phenol content, total antioxidant content, total anthocyanin content, and colour characteristics. Anthocyanins may have been the main antioxidants in red amaranth leaves based on the strong positive correlations between bioactive phytochemicals (TPC and TANC) and antioxidant activity (FRAP). As more people become interested in global sustainability issues, consumers are also willing to sacrifice on cost. Industries are now thinking about bringing the finest of these items from scientific studies to market and have been able to resolve numerous concerns associated to storage and stability. This industry is currently dealing with two problems: high manufacturing costs and a small market. Additionally, consumers look for convincing evidence to influence their choices and switch from synthetic to natural ones. Industries must use research findings to create new goods and fund related research. Because the base colours of anthocyanin offer complementing advantages, using anthocyanin as a natural food colourant is more appealing to consumers. Utilise this natural color in kalakand is more attractive and better nutritional quality than the normal one.

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Summary

Red amaranth leaves are the excellent source of antioxidant. The chlorinated sample dried at 60˚C gave better quality product. Midilli's model was found to satisfactorily describe the drying behavior of red amaranth leaves which showed the highest R2value, lowest X2value and RMSE values. The statistical indicators showed that Midilli's model was the best model to describe the drying kinetics of red amaranth leaves.

In this study, six thin-layers drying models namely Newton, Page, Modified page, Henderson and pabis, Logarithmic and Midilli models were used to select a suitable form of the drying curve of red amaranth leaves. The experimental data were used to select the best fitted model to describe the drying kinetics. The best fitted models depended on the basis of lower sum squared errors (SSE) value and root mean square error (RMSE) and the higher value of correlation coefficient.

Values were obtained in Midilli model. The Midilli model described the drying curve of red amaranth leaves satisfactorily. The Midilli *et al.,* (2002) model gave the highest value of R^2 (0.9997). For the control, chlorinated sample and water blanched sample at 50^0C showed the lowest SSE (0.0014) and RMSE (0.015), the lowest SSE (0.0019) and RMSE (0.014) for chlorinated sample and the lowest SSE (0.0026) and RMSE (showed 0.016) for water blanched sample, respectively. At 80^0C temperature the Midilli's model showed the highest value of R^2 (0.99952), the lowest SSE (0.0002) and RMSE (0.009) for control, the highest value of R^2 (0.99952), the lowest SSE (0.000042) and RMSE (0.014) for chlorinated sample and the highest value of R^2 (0.99952), the lowest SSE (0.00017) and RMSE (0.006) for water blanched sample.

Result showed the activation energy of blanched and unblanched samples for temperature range from 50, 60, 70 and 80 ^{0} C was reported as 15.7913 and 20.9221 kJ/ mol respectively. Darvishi *et al.,* (2016) reported the activation energy of dill leaves was16.84 kJ/mol for temperature range from 40, 50, 60 and 70 ^{0} C. During water blanching water can be removed from the sample by less energy due to the cell rupture of skin. The activation energy of chlorinated sample is higher than the control and water blanched sample. These values are consistent with the present estimated D_{eff} values for red amaranth leaves.

The statistical indicator showed that the Midilli's model was the best fitted model to explain the drying kinetics of red amaranth leaves. Among all these models we can consider the Midilli's model is the best fitted model to explain the drying process of different pre-treated red amaranth leaves sample, because in most cases, it represents the lower RMSE value. When the drying

temperature is raised in thin layer drying process for treated and untreated red amaranth leaves, the drying time is diminished and the drying rate is enhanced. The Midilli's model is the best empirical mathematical model to represents the drying curve of thin layer drying process of treated and untreated red amaranth leaves sample for all the drying temperature. In thin layer drying process blanching applied as a pretreatment to reduce the drying rate of the leaves.

The Rehydration behaviour of a thin layer dried red Amaranth leaves were studied at various temperatures 38°C, 50°C, 60°C and 80°C. Three types of pretreated samples were used for this rehydration process: normal, chlorinated and processed. Pretreated samples were dried at 50°C, 60°C and 80°C temperature. The rehydration process of the dried red amaranth leaves were satisfactorily described by the Peleg's equation. According to the Peleg's equation rehydration temperature increases from 38°C to 80°C, then the rate of rehydration constant K_1 significantly decreases and the capacity constant K_2 varies with different temperatures of rehydration. The increase in rehydration ratio was significant only as temperature increased from 38°C to 80°C.The rehydration ratio is higher at 60°C temperature for dried chlorinated, processed and control samples. An increase in temperature above 60°C had an adverse effect on the final rehydration ratio value, and it is decreased with increasing temperature. It is indicated that during the rehydration process temperature-induced to change the product and the loss of solids.

Osmotic dehydration of pineapple cuboids were conducted to study the effect of sugar concentration of osmotic solution on mass transfer, weight reduction, vitamin-C, total phenol content and antioxidant property of samples pretreated with steam blanching and microwave heating. As treatment time went on, there was an increase in water loss, weight loss, and solids accumulation. The sample treated with 60°B experienced the highest mass transfer during the osmotic dehydration of pineapple cuboids, whereas the sample treated with 30°B experienced the lowest mass transfer. The pineapple cuboids immersed in 60°B sugar syrup and dried in a tray drier resulted maximum weight loss. Microwave heated samples dipped in 60°B sugar syrup showed better retention of nutritive value(total phenol content, vitamin C and antioxidant activity) as well as better color, texture, taste and mouth feel .According to the sensory analysis, the samples treated with 60°B solution received the highest acceptability for color, flavour, texture, mouth feel, and taste. Osmodried samples were stored for 3 months at ambient condition without any adverse effect on sensory and nutritional parameters.

Red amaranth leaves though rich in bioactive compounds are commercially important leafy vegetables but highly perishable in nature.MAP was applied to store this vegetables in fresh condition without addition of any chemical preservative .It was found that the total phenol, antioxidant activity and color of red amaranth leaves stored under normal atmosphere drastically decreased after 3 days however, samples stored under MA showed four times increase in selflife and acceptability. MA technique is ideal as it delays degradation of leaf pigment. A Statistical approach was made for modified atmospheric packaging of minimally processed red Amaranth leaves. Box-Behnken design was used to optimize the conditions of modified atmospheric packaging with respect to time, temperature and the ratio between the size of the packet and the weight of the samples. Second order polynomial quadratic model was used for this study. The experimental results were in good agreement with the proposed regression model with p value $p< 0.05$. The maximum selflife is 12.1515 days at 19.4231 ˚C temperatures and the ratio between the size of the packet and the weight of the sample is 0.0888221.

Appearance and quality of foods have been greatly influenced by food acceptance. Plant pigments have a tremendous deal of promise to replace many synthetic colouring agents because they are the best source of colour in nature. Anthocyanins are secondary plant metabolites that are currently gaining favour for use as natural colourants in the food industry. A significant challenge and current need is the creation of practical, affordable methods for preparing natural food colour and using it in foods. Due to customers' rising interest in the aesthetic, dietary, and safety aspects of food items, there is a greater need for natural pigments like anthocyanin to be used as alternative colourants in food products.Red amaranth contains anthocyanin pigments and is frequently produced in high biomass, so it attracts interest as potential substitutes for the well-known colourant from red amaranth. The aim of this study was extraction of natural color, identification, quantification and it's utilization in food industry. Extraction of anthocyanin from aqueous solvent, 1% methanolic HCL, 2% methanolic HCL,2% methanolic citric acid and 1% glacial acetic acid has first time reported in this study. High performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) and LC-MS were used to evaluate the extract and identify the pigment. The outcome demonstrates the natural color anthocyanin presenct in red amaranth leaves. This is totally non-toxic. This natural color was utilised in Indian sweet Kalakand.

Future scope of work

I. Preservation of Red Amaranth leaves by other preservation techniques

II. Extraction of natural color from red Amaranth leaves by edible solvent

III. Utilization of natural color from red Amaranth leaves in other food product

Annexure 1: Identification of sample (Red Amaranth Leaves) by BSI

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संख्या/No.: CNH/Tech.II/2019/78

दिनांक/Date: 12-03-2020

To,
Ms. Arjuma Sultana
Research Scholar,
Department Of Food Technology and
Biochemical Engineering,
Jadavpur University
Kolkata, West Bengal

Sub.: Identification of one plant specimen – reg.

Dear Ms. Sultana,

Please refer to your letter dated 11th December 2011 along with a plant specimen for identification. The specimen is incomplete bearing only vegetative plant parts, without any flower or fruit. The specimen has been tentatively identified as:

Sl. No.	Specimen No.	Scientific Name	Family
1	AS–01	**Amaranthus tricolor** L.	Amaranthaceae

A complete specimen in all respects, with vegetative as well as floral parts is necessary for authentication.

The receipt of ₹ 50/- (Rupees Fifty only) Receipt No. TR-5, C-138147 dated 17-12-2019 is enclosed herewith.

Your specimen is returned herewith.

Yours sincerely

12/3/2020

(K. KARTHIGEYAN)
Scientist –'E'
वैज्ञानिक 'ई' Scientist- 'E'
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Estimation of effective moisture diffusivity of Red amaranth leaves (*Amaranthus tricolor* L.) for thin-layer drying technology

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Sultana, A. and Ghosh, U. (2021). Estimation of effective moisture diffusivity of Red amaranth leaves (*Amaranthus tricolor* L.) for thin-layer drying technology. International Journal of Agricultural Technology 17(2):737-752.

Abstract Red amaranth leaves are the excellent source of antioxidant. The chlorinated sample dried at60^{0}C gave better quality product. Midilli's model was found to satisfactorily describe the drying behavior of red amaranth leaves which showed the highest R^2value, lowest X^2value and RMSE values. The statistical indicators showed that Midilli's model was the best model to describe the drying kinetics of red amaranth leaves.

Keywords: Mathematical modeling, Total phenol, Antioxidant, Moisture diffusivity, Activation energy

Introduction

Amaranths are the most important leafy vegetable of the lowland tropics of Africa and Asia, but are scarcely known in South America (Palada and Crossman, 1999). A tricolor has a variety of leaf colors such as white (light green), dark green, red, purple and variegated (Palada and Chang, 2003) Green and red amaranths are mainly used as leafy vegetables. Red amaranth is a wonderful vegetable with reddish veined dark green leaves or fully red to purple leaves, suitable for growing in warm weather, in which young leaves and stems can be harvested periodically. Red amaranth is also especially nutritious, rich in easily digestible minerals i.e., iron and calcium, as well as protein, vitamin C, and beta-carotene (Islam *et al.,* 2003) The vitamins and minerals present in plants as natural or synthetic antioxidants have been linked to removing harmful molecules called free radicals in the body to help fight against infection and other conditions, including cancer, coronary artery disease, muscular degeneration, and serious eye diseases (Dasgupta and De, 2007). Leafy vegetables are sources of polyphenols, which prevents many chronic diseases, including cancer, cardiovascular diseases, and diabetes, has been well

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RESEARCH COMMUNICATION

Rehydration kinetics of thin layer -dried red Amaranth (*Amaranthus tricolor* L.) leaves

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Abstract

The rehydration behaviour of a thin layer dried red Amaranth leaves were studied at various temperatures 38 ^{0}C, 50 ^{0}C, 60 ^{0}C and 80 ^{0}C. Three types of pretreated samples were used for this rehydration process: normal, chlorinated and processed. Pretreated samples were dried at 50 ^{0}C, 60 ^{0}C and 80 ^{0}C temperature. The rehydration process of the dried red amaranth leaves were satisfactorily described by the Peleg's equation. According to the Peleg's equation rehydration temperature increases from 38 ^{0}C to 80 ^{0}C, then the rate of rehydration constant K_1 significantly decreases and the capacity constant K_2 varies with different temperatures of rehydration. The increase in rehydration ratio was significant only as temperature increased from 38 ^{0}C to 80 ^{0}C.

Keywords

Red amaranth leaves, drying, rehydration, rehydration kinetics, Peleg's constant

Introduction

Amaranthus are the most important leafy vegetables of the lowland tropics of Asia and Africa. The nutritional quality of red Amaranth leaves is high, highly proteinaceous and rich in iron, calcium, vitamin-C and beta-carotene (11). Leafy vegetables are the sources of polyphenols, which prevent many chronic diseases, including cancer, cardiovascular disease and diabetes, has been well documented (12). Amaranth's leaves are an important source of antioxidants (13). Leafy vegetables are highly perishable. Hence, dehydration techniques are used to prevent nutritional losses. Drying prevents the texture, color and flavor losses of leafy vegetables due to low moisture content. Storage is very important to extend the self-life and get the best quality product. Now a day's dried foods are very trendy, and it's used as processed food or ready to- eat foods. Dehydrated products are mainly rehydrated when using the product. Rehydration or the water absorption characteristics is a process of moisturizing the dried product made by an abundant amount of water. Rehydration is influenced by some intrinsic factors such as chemical composition of the product, pre-drying treatment, dehydration process, moisture removal conditions and method of storage after drying. As well as some extrinsic factors like composition of dipping solution, condition of rehydration and temperature. During drying process the quality of the dehydrated products depends on the effects of chemical and physical changes in raw material. Dehydration and rehydration process is reversible when the pre-drying and post-drying product quality is almost the same. Immersion of dry material in water results in many changes due

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Studies on Impact of Processing on Physiochemical and Biochemical Properties of Osmodehydrated Pineapple (*Ananas comosus (L) Merr.*) Cuboid and Its Storage Stability

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Osmotic dehydration of pineapple cuboids were conducted to study the effect of sugar concentration of osmotic solution on mass transfer, weight reduction, vitamin-C, total phenol content and antioxidant property of samples pretreated with steam blanching and microwave heating. As treatment time went on, there was an increase in water loss, weight loss, and solids accumulation. The sample treated with 60°B experienced the highest mass transfer during the osmotic dehydration of pineapple cuboids, whereas the sample treated with 30°B experienced the lowest mass transfer. The pineapple cuboids immersed in 60˚B sugar syrup and dried in a tray drier resulted maximum weight loss. Microwave heated samples dipped in 60˚B sugar syrup showed better retention of nutritive value(total phenol content, vitamin C and antioxidant activity) as well as better color, texture, taste and mouth feel .According to the sensory analysis, the samples treated with 60°B solution received the highest acceptability for color, flavour, texture, mouth feel, and taste. Osmodried samples were stored for 3 months at ambient condition without any adverse effect on sensory and nutritional parameters.

Keywords: Osmodehydration; pineapple; kinetic study; bioactive compound; storage stability.

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Identification of Aqueous Extract of Red Amaranth Leaves by HPLC and LC-MS

Arjuma Sultana and Uma Ghosh

Abstract The demand for natural antioxidants in the upcoming years is set to rise exponentially due to increasing consumer awareness of harmful effects of synthetic antioxidants on human health. Fruits and vegetables are rich sources of bioactive compounds having antioxidant property. The natural dietary antioxidants of fruits and vegetables promote health effect. Various flavonoids and phenolic acids present in fruits and vegetables are responsible for prevention of several chronic diseases including cardiovascular disease, inflammation, and cancers due to their antioxidant properties. Red amaranth looks wonderful with purple or fully red in color. It is a good source of nutrients with high-quality proteins, vitamins, minerals, fibers, and bioactive compounds such as phenolics and pigments. Red amaranth leaves are the rich source of polyphenol and antioxidant. They are also helpful to increase the awareness of consumers in regards to the benefit of phyto-chemical. Solvent extraction is one of the most common conventional methods for extraction of color from leafy vegetables. The aim of the present study was to apply the conventional aqueous extraction procedure in extracting the color of red amaranth leaves. The aqueous extract was analyzed by high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) for identification of the pigment. The result reveals the presence of anthocyanin pigment in red amaranth leaves.

Keywords Red amaranth leaves · Bioactive compounds · Antioxidant · HPLC · LC-MS

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