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PHYTOCHEMICAL AND MICROBIOLOGICAL ANALYSIS OF DEVELOPED FREEZE DRIED WATERMELON AND TOMATO POWDER

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ABSTRACT

Fruits and vegetables are the prominent source of various nutrients as well as phytochemicals. Due to their higher water activity, they are prone to deterioration. The present study was conducted to prepare freeze dried powder mix of Watermelon(*Citrullus vulgaris*) and Tomato(*Lycopersicon esculentum Mill*).Freeze drying or lyophilization is a method of food dehydration to make high quality food products without altering their colour, texture, flavour and nutritive contents. In this study, the phytochemical(qualitative and quantitative),microbiological and antioxidant capacity and lycopene content have been evaluated. For this analysis the variations of 10%,20%,30%,40% and 50% were prepared. Result shows a gradual increase in TPC, antioxidant capacity and lycopene content. The microbiological (bacterial and fungal) load for each variation was found to be constant for 45 days during 15 days interval.

1.Introduction

Fruits and vegetables are the essential sources of vitamins,minerals,fibers and other phytochemicals.A regular consumption of these shortens the risk of chronic diseases.Watermelon belongs to the Cucurbitaceae family, which include melons,cucumbers and pumpkins.Watermelon (*Citrullus vulgaris*) is widely cultivated throughout India . The sweet pulpy flesh of watermelon is highly refreshing and consumed fresh. The watermelon juice forms a cooling and refreshing beverage highly valued during summers. Juice is reported to have diuretic properties and is a good source of potassium and other minerals.Lycopene, a red pigment of the carotenoid class found in only a few fruits and vegetables, is a powerful oxygen radical scavenger and highly effective antioxidant . Watermelon and tomatoes are the most familiar sources of lycopene in the Western diet, containing on average 48.6 and 30.1 µg lycopene/g fresh weight, respectively.The amino acids citrulline and arginine have been studied widely by medical researchers for their usefulness in sickle cell anemia, immune function, wound healing, and cardiovascular health (Watermelons and Health January ;2007).

Tomato (*Lycopersicon esculentum Mill*) belongs to family "Solanaceae" and is a worldwide important agricultural commodity..In terms of area, tomato is the second horticultural product cultivated and the first in industrilized volume.(Filhoz C,et al.;1996).Tomato is a climacteric fruit,with a short shelf-stability under ambient storage conditions.(Shahnawaz M.,et al.; 2012).Drying is the most suitable method to comply with the above requirements. Dried tomato products are used as important ingredients for pizza, various vegetables, spicy dishes.Tomato is an important vegetable crop which grows worldwide. During their peak

season farmer doesn't get good price moreover a big share of crop produce is spoiled and become a waste due to lack of proper processing and storage facilities. However, it can be converted in to some value added products to achieve the greater value in the market .By drying to a certain moisture level the dried fruit and vegetable powders can be an ideal addition to soups, sauces, marinades, baby foods, dips, extruded cereal products, fruit purees, and fillings for frozen toaster snacks. (Srivastava,S,et al.;2013).The antioxidant lycopene is the most abundant carotenoid in tomatoes,which is the point of increasing interest. Presence of lycopene promoted research activities on fresh tomatoes and tomato products.(Bashir, N, et al.;2014).In recent years, the interest is inclining towards the use of these elements as micro-nutrient supplements or functional foods in medical treatment to prevent various diseases such as cancer,cardiovascular diseases, AIDS, Alzheimer's disease, osteoporosis, osteoarthritis, asthma, cataract, fatigue and ageing (Hunt, C.D;1996) .Freeze drying also known as Lyophilization is one of the techniques of dehydration which is suitable for the preparation of high value dried products.In this technique, the product quality did not undergo any change (color, shape, aroma and nutritional value), which is higher than other products obtained by different drying techniques. The method works at low temperature and occurs in the lack of oxygen which help in reducing the degradation reaction (Litvin et al.1998). The freeze dried material are hygroscopic and can be rehydrated more quickly and completely then other dried samples (Giri ,S.K.,et al;2007). There is no such loss of bioactive compounds in this method like flavonoids, flavonols, flavones,catechins and phenolics (Asami ,D.K.,et al, 2003).In freeze drying the mechanism of ice sublimation under low pressure Freeze drying is a costly and labor intensive process but retains the flavor, taste and color of the product when re-introduced into the water.Vegetable and fruit juice powders have many advantages and economic potentials over their liquid counterparts such as reduced volume or weight, reduced packaging, easier handling and transportation, much longer shelf life and nutritional supplements (Pradyuman Kumar (2018). Freeze drying has been widely used in a number of applications for many years. It is commonly used in the food and pharmaceutical industries. There are, however, many other uses for the process including the stabilization of living materials such as microbial cultures, preservation of whole animal specimens for museum display, restoration of books, stabilization of perishable food products by dehydration and other items damaged by water, and the concentration and recovery of reaction products.

2. Materials and method.

2.1.Preparation of sample: For the preparation of dried fruit powder the watermelon and tomatoes were procured from the local market of Gwalior(Madhya pradesh).The fresh red and ripened tomatoes without any bruises were selected for the sample preparation.The sample was properly washed with clean potable water (with 1% NaCl solution for surface cleaning). The watermelon was cut to remove the rind and converted in to small pieces.The samples were frozen at -35°C for 24 hrs and dried under vacuum condition at -20°C for 24 hrs(only for tomato) and 48 hrs (for watermelon).The freeze dried samples were packed into HDPE pouches and stored at normal conditions.

2.2.Working sample preparation: For further analysis the prepared samples were taken in an appropriate quantity and mixed with butanol,methanol and water. The samples were kept on orbital shaker for 24 hrs and filtered by whatsmann filter paper no.4 to be used for further analysis.

2.3.Evaluation of secondary metabolites:

2.3.1.Qualitative analysis for tannin:

Crude extract was mixed with 2 ml of 2% solution of Ferric Chloride. A blue-green black

colouration indicated the presence of phenols & tannins. (1ml sample + 500µl FeCl3)

2.3.2.Qualitative analysis for saponins:

Dried powder (1mg.) was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 seconds. 1cm layer of froth stable for 1 min indicates the presence of saponin. (1ml sample + 5ml distilled water).

2.3.3.Qualitative analysis for flavonoids:

Extract were treated with few drops of sodium hydroxide solution. Formation of yellow colour which become colourless on addition of dilute acid, indicate the presence of flavonoid. 10% NaOH solution (2ml) + 100 µl sample gives yellow colour.

2.3.4.Qualitative analysis for Alkaloids:

To 1 ml of extract, 2-3 drops of Wagner's reagent were added. The appearance of pale or white precipitate indicated the presence of alkaloids.

2.3.5.Qualitative analysis for phenolics :

To 1m.g of freeze dried sample, 2 ml of distilled water and few drops of 10% ferric chloride solution were added. Formation of blue or green colour indicates the presence of phenols.(Johri S.,et al.;2017).

2.3.6.Quantitative estimation of TPC content and Antioxidant activity:

Total Phenolic Content:

The total phenolics in the samples were determined using Folin-Ciocalteu method (Habila J D ., et al.;2010). To each sample solution (1.0 ml) and the standard (gallic acid) was added 5 ml of folin-ciocalteu (sigma-aldrich) and 4 ml sodium carbonate (7% w/v) and shaken. The solution could stand for 30 min in the dark at room temperature, after which absorbance was measured at 765 nm using spectrophotometer (UV-VISIBLE parkin elmer Lambda 25). The phenolic content was calculated from the standard curve of gallic acid.

$$C = (c \times V)/ m \quad (1)$$

Where,
C = total content of phenolic compounds mg/gm plant extract
c = the concentration of gallic acid established from the calibration curve (mg/ml)
V = the volume of extract in ml.
m = weight of sample in g.

Reducing power estimation :

The reducing power of methanolic extracts increases with the increased in concentration. The reducing power was best observed at 1mg/ml concentration. The presence of reducers (i.e antioxidants) causes the reduction of Fe3+ or ferricyanide complex to the ferrous form. Therefore, measuring the formation of Prussian blue at 700 nm gives an indication of Fe2+ concentration and the reducing capability (Chauhan N.,et al.; 2019).

The electron donating capacity of the bioactive compounds is defined as the reducing power or the antioxidant activity. The antioxidant property of a compound or extract could be described as a redox reaction in which a reactant species (antioxidant) is reduced by the exposure of the oxidant. Different fraction of aqueous plant extract at various concentration (200 - 1000µg/ml) were prepared.2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% potassium ferrocyanide was mixed in 1 ml of different extract prepared. The test tubes were incubated in water bath for 10 minutes at 50°C followed by addition of 2.5 ml of 10% TCA and was centrifuged at 3000rpm for 10 minutes. 2.5 ml of upper layer obtained was collected and mixed with 2.5 ml of distilled water followed by addition of freshly prepared 0.5 ml of 0.1% FeCl3. Absorbance was noted at 700nm against a suitable blank.(Ijaz F.,et al.;2017)

Nitric oxide scavenging activity :

Extract of different dilutions (0.1 to 1 mg/ml) dissolved in PBS (25mM, pH7.4) were prepared. 200µl sodium nitroprusside (5mM) was added to 800µl of the prepared dilutions. The mixture was then incubated at 37 °C for 2.5 hours under normal light followed by incubation of 20 minutes in dark. 600µl Griess reagent was added followed by incubation for 40 minutes at room temperature and absorbance was noted at 540 nm against a suitable blank(Chauhan N.,et al.; 2019)and

percent of inhibition was calculated by using this equation:

% Inhibition = (OD of control- OD of extract/OD of control)×100. (2)

Total Antioxidant Activity:

Different dilutions of 1 mg/ml of freeze dried powder were prepared and to it 4 ml of 28 mM sodium phosphate, 4mM Ammonium molybdate and 0.6 M Sulphuric acid was added and were put in capped test tube and were left for incubation in a water bath for 90 min at 95℃. The incubated sample was thereafter cooled to room temperature and the absorbance was measured at 695 nm against blank. Antioxidant activity was expressed relative to that of BHT which was used as standard (Capek I.;2004).

Lycopene content estimation of freeze dried powder:

Lycopene has absorption maxima at 473 nm and 503 nm.The standard procedure has been followed to estimate the lycopene content in freeze dried powder. For the analysis 5 gm.of sample was taken.samples were extracted repeatedly with acetone in pestle and mortar until the residue became colourless.The mixture was transferred to a separating funnel containing 10-15 ml. Of petroleum ether.To the petroleum ether extract small quantity of anhydrous Na_2SO_4 was added.The collected extracts were measured by Spectrophotometer (Perkin elmer uv vis, lambda 25) at and 473 nm and 503 nm (Slightly modified) using petroleum ether as blank (Rangana S.;2011).

$$mg\ of\ lycopene\ per\ 100\ g = \frac{3.1206 \times OD\ of\ sample \times Volume\ made\ up \times 100}{1 \times Wt\ of\ sample \times 1000} \quad (3)$$

Statistical Analysis: All data were reported as mean and standard deviation of three measurements.

Microbial load:

Microbiological analysis included isolation,enumeration and identification of bacteria and fungus using standard protocol. Media used for this analysis included nutrient agar, peptone water,EMB agar, mannitol salt agar and sabouraud dextrose agar.For isolation and enumeration of pathogens in samples, lyophilized powder of different concentration is used with 9ml.of sterile peptone water.The 10-fold serial dilution was performed and 0.1ml.of last two dilutions(10^{-4} and 10^{-5}) were inoculated on desired media using plate count technique. Spread plate technique was done by inoculating 0.1ml of the appropriate dilutions on plate count agar plate for enumeration of bacteria and on potato dextrose agar for fungal count. The agar plates were incubated at 30ºC for 24-48h for bacterial count and at 26ºC for 3-5 days for fungal count. Each sample was inoculated in duplicate agar plates and the mean values of bacterial and fungal counts were recorded as colony forming unit per ml (cfu/ml) (Braide W.,et al.;2014).

3.Results and discussions

3.1.Qualitative estimation of secondary metabolites

Secondary metabolites are numerous organic molecules produced by plant cells through metabolic pathways.These metabolites possess various biological effects.For the present study the qualitative estimation has been carried out for tannins, saponins, flavonoids, phenolics, alkaloids and terpenoids .The methanoic and hot aqueous extractions have been prepared for analysis.Result shows difference in both the of extracts.

Table 1. Qualitative phytochemical screening of various dilutions of freeze dried powder**.**

Qualitative tests	**Methanol extract**	**Hot aqueous extract**
Tannins	++	+++
Saponins	+	++
Phenolics	+++	+++
Flavonoids	++	+++
Alkaloids	++	++
Terpenoids	+++	++

(+)Fairly present , (++) moderately present,(+++) highly present, (-) indicates absence of compounds

Table 2. Quantitative estimation of TPC content and antioxidant activity of various dilutions of freeze dried powder.

%Compositions	**TPC content (GAE/gm.)**			**Reducing power(%)**			**Total Antioxidant Activity(%)**		
Extraction	Butanol	Methanol	Aqueous	Butanol	Methanol	Aqueous	Butanol	Methanol	Aqueous
10%(VFI)	592.5±0.577	593.15±2.624	550.27±0.573	0.351±0.002	0.271±0.010	0.161±0.003	0.107±0009	0.200±1.055	0.100±0.134
20%(VFII)	573.75±0.500	582.4±0.969	454.02±2.085	0.425±0.023	0.310±0.416	0.192±000	0.147±0.018	0.110±0.008	0.122±0.164
30%(VFIII)	460.25±0.500	511.37±1.065	420.25±0.465	0.44±0.014	0.352±0.001	0.26±0.004	0.307±0.412	0.212±0.322	0.247±0.006
40%(VFIV)	421.25±0.957	497.75±0.506	353.67±0.855	0.52±0.016	0.444±0.001	0.363±0.004	0.327±0.005	0.277±0.416	0.320±0.429
50%(VFV)	372±0.816	421.97±0.05	321.5±0.298	0.617±0.828	0.607±0.002	1.455±0.488	0.355±0.01	0.310±0.008	0.337±0.005

*VF=Vegetable and fruit powder

*Data expressed as a mean±standard deviation for three experiments.

Table 3. Nitric oxide scavenging activity.

%Compositions	Nitric oxide scavenging activity(%)		
	Butanol	Methanol	Aqueous
10% (VFI)	0.727±0.01	0.632±0.009	0.501±0.000
20% (VFII)	0.612±0.001	0.5815±0.004	0.490±0.001
30% (VFIII)	0.548±0.004	0.435±0.000	0.435±0.584
40% (VFIV)	0.439±0.004	0.375±0.001	0.3522±0.002
50% (VFV)	0.375±000	0.281±0.377	0.294±0.395

*VF=Vegetable and fruit powder

*Data expressed as a mean±standard deviation for three experiments.

Table 4. Quantitative estimation of Lycopene content

S.no	% Compositions	Lycopene content (mg/100g) absorbance at 503nm	Lycopene content (mg/100g) absorbance at 473 nm
1.	10%(VFI)	10.656±0.007	9.506±0.228
2.	20%(VFII)	10.812±0.006	12.588±0.013
3.	30%(VFIII)	12.107±0.053	13.389±0.355
4.	40%(VFIV)	12.529±0.005	14.616±0.014
5.	50%(VFV)	12.794±0.002	14.664±0.004

*VF=Vegetable and fruit powder

*Data expressed as a mean±standard deviation for three experiments.

Table 5. Microbiological analysis of Freeze dried fruit powder

Storage (days)	VFI		VFII		VFIII		VFIV		VFV	
	Bacterial Isolates	Fungal Isolates	Bacterial Isolates	Fungal Isolates	Bacterial Isolates	Fungal Isolates	Bacterial Isolates	Fungal isolates	Bacterial Isolates	Fungal Isolates
0	-	-	-	-	-	-	-	-	-	-
15	$0.12X10^5$	$0.09X10^5$	$0.12X10^5$	$0.10X10^5$	$0.14X10^5$	$0.10X10^5$	$0.16X10^5$	$0.10X10^5$	$0.16X10^5$	$0.10X10^5$
30	$0.12X10^5$	$0.09X10^5$	$0.12X10^5$	$0.10X10^5$	$0.14X10^5$	$0.10X10^5$	$0.16X10^5$	$0.10X10^5$	$0.16X10^5$	$0.10X10^5$
45	$0.12X10^5$	$0.09X10^5$	$0.12X10^5$	$0.10X10^5$	$0.14X10^5$	$0.10X10^5$	$0.16X10^5$	$0.10X10^5$	$0.16X10^5$	$0.10X10^5$

***VF=Vegetable and fruit powder**

3.2.Quantitative estimation Total Phenolic content

The total phenolic content was calculated as mg. Gallic acid equivalent (GAE) of freeze dried powder.The extract was made in butanol, methanol and aqueous solution.As results shown in Table:II,the total phenol content was obtained in the range of 592.50±0.577(10%composition) to 372.00±0.816(50%composition) in butanoic extract.In metanoic and aqueous extracts the phenolic content was analyzed from 593.115±2.624 to 421.97±0.05 and 550.27±0.573 to 321.50±0.298 (in 10-50% composition) respectively.The result in Table:II shows five respective formulations, the total phenolic content decreases with the percent of watermelon percent in the composition.The highest percent of total phenol has been observed in methanoic extract and lowest in aqueous extract.

3.3.Reducing power estimation

The electron donating capacity of a bioactive compound is defined as reducing power.The reducing power of freeze dried powder was measured by preparing butanoic,methanoic and aqueous extracts.It was found that the butanoic extract had the highest reducing power while the aqueous extract had the lowest.In the results Table:II, the reducing power was found from 0.351±00.02 to 0.617±0.828 in butanoic extract,0.271±0.010 to 0.607±0.002 in methanoic extract and 0.161±0.003 to 1.455±0.488 in aqueous extract respectively.

3.4.Nitric Oxide scavenging activity :

Nitric Oxide reacts with oxygen to produce stable products like nitrate and nitrite,under aerobic conditions.By using Griess reagent we can determine the quantities of them.Free radical scavengers are substance like antioxidants,that help to prevent cells from being damaged by free radicals.As above the extracts were prepared in different solvents.From observations shown in Table:III, the increase in concentration leads to decrease in absorbance for all the five variations.Therefore it can be presumed that tomato and watermelon have Nitric Oxide scavenging activity.

3.5.Total antioxidant capacity and Lycopene content:

The total antioxidant capacity is the capacity of an antioxidant to suppress the harmful effect of free radicals in blood and cells.The results of total antioxidant capacity can be observed in Table:IV(observations in percent inhibition),For extraction butanol,methanol and distilled water were taken as solvents.The antioxidant capacity of different compositions was found in increasing order.The highest value was found in butanoic extract and lowest in methanoic extract.The range was observed from 0.107±0.009 to 0.355±0.01(butanoic extract),0.200±1.055 to 0.310±0.008 (methanoic extract) and 0100±0.134 to 0.337±0.005 (aqueous extract) respectively.

From the above observations it can be concluded that the antioxidant capacity is due to the presence of lycopene content and other secondary metabolites present in tomato as well as in watermelon.

Lycopene is a red pigment generally found in fruits and vegetables. It is a carotenoid pigment which has antioxidant properties.Fresh tomato or tomato products and watermelon both are prominent sources of lycopene pigment.The results shown in Table:IV,a gradual increase was found in different compositions.

The highest content of lycopene was observed in 50% composition (12.794±0.002).The lycopene content of freeze dried powder varied in the range of 10.656±0.007-12.794±0.002 respectively at 530nm.At 473nm. The observations were noted in the range of 9.506±0.228 -14.664±0.004. It can be stated that with increase in tomato powder the lycopene content also increases.A transmittance spectrum of lycopene in petroleum ether acquired by the Spectrophotometer (Perkin elmer uv vis, lambda 25) 473 nm and 503 nm, and the maximum absorbance was at 473 nm. (Davies, 1976).

3.6.Microbiological analysis

For microbiological study standard procedures have been followed to identify bacterial and fungal load.The microbiological

analysis is a tool to establish the relationship between development and handling of the finished product to identify minimal load of microorganisms.The standard load count has been observed at a duration of 15 days(the observations has been taken for 45 days).Number of bacteria and fungus were calculated per ml.or grams of sample by dividing the number of colonies by dilution factor.Colony forming unit(cfu) is a measure of viable bacterial and fungal cell.As shown in Table:V the bacterial count at 15 days interval remained constant ($0.12X10^5$) for variation I, ($0.12X10^5$) variation II,($0.14X10^5$)Variation III, 0.($16X10^5$) Variation IV and ($0.16X10^5$) Variation V.The fungal count at 15 days interval also remained constant. It was found ($0.09X10^5$)Variation I, ($0.10X10^5$) Variation II, ($0.10X10^5$) Variation III,($0.10X10^5$) Variation IV and ($0.10X10^5$) V till 45 days.

4.Conclusions

Food preservation is an essential process to increase the shelf life of food.Fruits and vegetables are susceptible to deterioration due to the presence of high water activity.Freeze drying or lyophilization is a technique to suppress the water activity by sublimation of water.As compared to other drying techniques this method applies low temperature and pressure which automatically reduces the chance of reduction in nutritional as well as esthetic qualities of food product.In the present study the observation shows that the freeze dried powder is containing saponins, flavonoids, tannins, phenols, alkaloids and terpenoids respectively.

The quantitative analysis has also been done which evaluates the moderate quantity of secondary metabolites and antioxidant activity.Tomato and watermelon both are rich in lycopene content naturally,which has been reported in this study.This can be concluded that such powders can be consumed by different age groups which may help to bring natural health benefits to the society.

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