Original Research

Extraction of beta carotene and analysis of different components from carrots by physicochemical methodology
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Abstract

Background: Carrots encompass numerous beneficial effects on the human being. Objective: Carrots are worn as groceries and we also intake a sap of carrot which produces superior upshot scheduled the liver and as well stable for vision. Carrots are a font of reserves, vitamins, carbohydrates, proteins, fats, fibers, and vigor which is the mainstream imperative for vigorous and strapping vigor structure. Method: The font of vitamin which is the most imperative element of our venture is beta carotene and carotenoids but our foremost focal point is lying on beta carotene, Qualitative scrutiny of diverse component, and in quantitative scrutiny of moisture content. Carbohydrates and proteins are obligatory for corpse vigor and mane escalation, covering and nails escalation respectively. Beta carotene is an antioxidant that prevents the oxidation of an assortment of compounds hips the corpse. This manuscript presents an appraisal of the widespread scrutiny of diverse gear's lip carrots and the withdrawal of beta carotene. Results: Carrots of two diverse kinds were serene (red and orange) and analyzed qualitatively by physicochemical slant and the outcome showed that the quantity of beta carotene was diverse hip the entire carrots and moisture content be as well diverse (red carrot 90.3% and hip orange carrot 93.7%). Conclusion: Carbohydrates and proteins were present in hip the entire carrots and severance of beta carotene using diverse non-polar solvent (acetone, chloroform, dichloromethane, n-hexane) gave detach results.

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Introduction
Carrot (Daucus carota L.), herbaceous species, in the main biennial vegetation is a member of the Apiaceae family [1]. Including 39 fruits and vegetables, carrots gained 10th position in nutritional value [2]. The carrot edible taproot is a good source of carotenoids, vitamins, carbohydrates, proteins and dietary fiber along with minerals and antioxidants have become important [1]. Carrot roots contain 88% water, 1% protein, 7% carbohydrate, 0.2% fat, and 3% fiber along with vitamins (antioxidants) and minerals [1].

Now, orange carrots have gained great attention and are cultivated in the world in a wide manner [1]. Yellow, orange, and red carrots are composed of carotenoids, beta carotene, and richest in antioxidants (provitamin A) [3]. Orange carrot now a days widely used everywhere contains a high percentage of α- and β-carotene and rich in font of provitamin A. The taste of carrots depends on glutamic acid and the buffering action of free amino acids [2]. Record on the flavor profile of eight carrot withdrawal from the levels of volatile Terpenoids which shows that greater than 90 volatile compounds present in carrots that provide them their characteristic flavors. There is big bioaccumulation of vitamin A and beta carotene in carrots which are useful to improve eye healthfulness [3]. The yellow color of carrot is dependent on lutein which helps in the prevention of macular degeneration.

The yellow, orange, and red colors of carrots are due to the moderately big dissolve nutrient carotenoids. Anthocyanins defined as a group of polyphenols pigments play an important role to give carrots a purple color. Anthocyanins are responsible for photo protection, scavenge free radicals and the most important attraction of animals for pollination in plants [4, 5]. For health promotion and protection from cardiovascular disease [6, 7] and cancer [3] by acting as dietary antioxidants, reducing inflammation and lipid oxidation, causing induction of anti-inflammatory and vasoprotective effects, phase II enzymes, and apoptosis dietary anthocyanins play a major role. Anthocyanins implications produce considerable effects in improving brain and memory functions [8].

All these pigments have been considered due to fitness comfort belonging to them as well as defend from specific malignant and congestive heart failure, and user concern with every common diet abundant with these compounds, often say ‘functional foods’ is rising carrots [9]. Basically, the color of red carrots is due to the lycopene which the carotenoids. Provitamin A task cannot show by lycopene although it is a hydrocarbon. It is the largest powerful antioxidant in artificial insemination of all the carotenoids that originate as in much as in humans [10].

Carrots are also a good source of carbohydrates and minerals like Ca, Fe, Na, K, Mg, Cu, Zn, carotenes, thiamine, riboflavin, niacin, vitamin C [11] [5] [4]. The carbohydrate contains simple sugars, like sucrose, glucose, and fructose, along with a small quantity of starch [1] Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimitogenic and antitumor activities. They have been reported to be a potential scavenger of free radicals, which are harmful to our body and food systems [12]. Although Phenolics compounds do not have any known nutritional function, they may be important to human health because of their antioxidant potency [13] [5] [4].

Phenolics or polyphenols because of their physiological effect like antioxidant, antimitogenic, and anti-tumor activities have gained considerable attention. For body and food system protection Phenolics or polyphenols act as a potential contender to combat free radicals because free radicals are dangerous to our body and food systems [12] [4]. Carrot intake does not provide a sufficient amount of calories to the human diet but provides nutrition in Phytochemicals, such as carotenoids, anthocyanins, and other Phenolics compounds [1].

Higher recurrent, polyacetylene recognized in carrot has been included as bioactive compounds with optimistic reflex on biped genetics and distemper [14]. Homologous several further chromism herbaceous plants, the carrot is a valuable resource of antioxidants. Carotenoids, polyphenols, and vitamins exist in carrots operate as antioxidants, and carcinoma and immunodiagnostics. Carotenoids extensively divided in orange carrots are influential antioxidants that can negate the reaction of free radicals. They have been exposed to have restricted transformation motion accompanying to diminish the danger of some carcinoma [2].

B carotene (the carotenoids with the most provitamin A activity) in carrots helps to protect vision, especially night vision, and also provides protection against macular degeneration and development of senile cataract, the leading cause of blindness in aged people [2]. Eating carrots rich in β-carotene may restore vision, lending truth to the adage that carrots are good for your eyes. Carrots are one of the richest sources of provitamin A and a high intake of carotenoids linked with a significant decrease in postmenopausal breast cancer [2].

Research has shown also that smokers who eat carrots more than once a week have a lower risk of lung cancer [2], while a β-carotene-rich diet may also protect against prostate cancer [2].

Carrot digestion possibly includes strengthening of the immune system; depend on opposed Knocks, steeped blood pressure, osteoporosis, cataracts arthritis, heart defects, bronchial asthma, and urinary tract sickness [15-17]. Carotenoids moreover operate as a free radical hunter and are more imperative for health [18, 19]. D’Odorico et al have exposed that the existence of α and β carotene in blood has a defensive impact opposed atherosclerosis [20]. Nocelle et al has exposed those giant carotenoids calories are linked with a decreased hazard of heart defects [21]. Phenolics or polyphenols have gained appreciable consideration because of their genetic tasks, containing antioxidant, antimitogenic, and antitumor actions [22]. They have been announced to be an optimistic competitor to fight free radicals, which are unsafe to our body and food system [12] [4]. Carotenoids have been recognized as an optimistic barrier of Alzheimer’s disorder [23].

Experimental Section
The vegetables used for the study are orange carrot (Daucus Carota), and red carrot (Daucus Carota).

Materials and Methods

Saleem et al.
The research study was approved out at the Women University of Bahawalpur and the sample was processed in the analytical laboratory of GSCWU.

Major equipment used during the experiments is electric balance, separating funnel, beakers, thermometer and pipette, spatula, tripod stand, wire gauze, aluminum foil, 110 thermometers, and the most imperative water bath to carry out the reaction at a constant temperature.

The foremost techniques used for extraction of beta carotene are:

**Soxhlet extraction:** In this technique, a finely ground sample reacted in a suitable solvent. Frequently organic solvents are used.

**Decoction extraction:** In this technique, plant materials are boiled in water to collect the extract. The solvents used are water or alcohol.

**Sample collection:** The 2 different kinds of samples were collected from different shops and home cultivated.

**Sample processing:** Samples were processed on the same day of sample collection.

**Analysis methods:**

**Extraction of beta carotene from carrots**

In 30g ground, carrots added 185ml of ethanol and 10ml water. Heated it in a water bath with a maintained temperature at 45°C for one hour. Then cooled the exceeding solution. Then filtered it. Took 10ml of extract and 10ml of solvent. Miscellaneous them beta carotene layer separated from the solvent due to immiscibility and then separated both layers. Left that separated content for few days and after that, we observed that liquid removed out from beta carotene and it came in powdered form which was yellow. Pictures revealed under correspond to beta carotene in powder form.

Diagrams showed in this part of the dissertation signify the solvent layer that has been separated from the beta carotene layer but from those solvents which were immiscible. The beta carotene layer cannot be extracted from that solvent which was miscible like NH3 solvent, it showed miscibility with beta carotene and solution converted into green color with precipitate formation.

The diagrams shown below are beta carotene and solvents which have been separated after mixing both of them.

**Qualitative detection method**

**Taste:**

- Red carrot: sweet
- Orange carrot: less sweet

**PH:**

- Red carrot: neutral
- Orange carrot: neutral

**Moisture content**

- Red carrot: 90.3%
- Orange carrot: 93.7%

**Color of carrot extract with methanol**

- Red carrot: yellow
- Orange carrot: light yellow

**Color of dried carrots after evaporating the moisture**

- Red carrot: Dark brown
- Orange carrot: Dark brown

**Test for carbohydrates:**

**Confirmation of Glucose**

In a carrot extract minute quantity of Benedict reagent was added and heated in a water bath and renamed the color change.

In a carrot extract small quantity of Fehling solution was added and heated it in a water bath and renamed the color change.

**Confirmation of sucrose**

In a carrot extract added few drops of HCl. Heated it for two minutes. Then Added NaOH and benedicts reagent and again heated. Renowned the color change, which showed the presence of sucrose.

**Confirmation of starch**

In a carrot extract added few drops of iodine solution. Renowned the color change, which showed that starch, is present in both kinds of carrot extract (red and orange carrot).

**Test for tannins**

Took 10ml of extract and poured 2ml of 5 % ferric chloride in it. Renowned the color change.

**Test for Coumarins**

Tool 1 ml of extract and poured 1ml of 10% sodium hydroxide in it. Renowned the color change.

**Test for Amino acid (Ninhydrin test)**

Tool 2ml of extract and injected the minority drops of 0.2% ninhydrin reagent. Then warmth for 5 minutes. Renowned the color change.

**Test for Quinones**

 Took 1ml of extract and poured 1ml of concentrated Sulphuric acid. Renowned the color change.

**Test for Saponins**

2ml of extract and 2ml of distilled water were assorted and waggled in a graduated cylinder for 15 minutes lengthwise. Renowned the color change.

**Test for Terponds**

Took 0.5ml of extract and poured 2ml of chloroform and conc.H2SO4. Renowned the color change.

**Test for Phlobatannins**

Took 1ml of extract and infused a few drops of 2% HCl in it. Renowned the color change.

**Test for phenols**

Took 1ml of carrot extract and poured 2ml distilled water. Then injected the minority drops of 10% ferric chloride in it. Renowned the color change.

**Test for steroids**

Took the equivalent quantity of extract and chloroform. Then added the minority drops of concentrated H2SO4 in it. Renowned the color change.

**Test for Flavonoids**

Took 2ml of extract and poured 2N NaOH in it. Renowned the color change.

**Biuret Test**

Took a 2ml precipitate solution. Then Added 2ml 10% NaOH in it then added 2-3 drops of copper sulfate. Diverse it tenderly. Renowned the color change.

**Test for Anthracanine**

Took 1ml of extract and poured 1ml of 2N NaOH and warmth for 5 minutes at 100°C. Renowned the color change.

**Test for Glycoside**

Took 2ml of extract and 3ml of chloroform was poured in it. Then 10% NH3 solution was added. Renowned the color change.
Quantitative analysis

Determination of moisture content:

Determine moisture content in different carrots

Red carrots:

Moisture content can be calculated by using this formula:

\[
\text{percentage moisture content} = \frac{W_1 - W_2 \times 100}{W_2} \times 100
\]

Where;

- \(W_1\) = wt. of fresh sample + China dish = 35.93g
- \(W_2\) = wt. of dried sample + China dish = 32.73g
- \(W\) = wt. of fresh carrot = 3.54g

\[
\text{percentage moisture content} = \frac{35.93 - 32.73}{3.54} \times 100 = 90.3\%
\]

Orange carrots:

- \(W_1\) = wt. of fresh sample + China dish = 3.346g
- \(W_2\) = wt. of dried sample + China dish = 3.003g
- \(W\) = wt. of fresh carrot = 3.66g

\[
\text{percentage moisture content} = \frac{3.346 - 3.003}{3.66} \times 100 = 93.7\%
\]

Results and Discussion

Carrots restrain a huge quantity of beta carotene which is extremely imperative for our vigor. It lowers the threat of colon cancer, according to 2014 research that incorporated facts from 893 populace. So, the main focus was on the extraction of beta carotene. Beta carotene structure consist of combination of cyclohexene and many dienes.

From carrots, huge quantities of calories are obtained. Water, carbohydrates, proteins, fiber, and beta carotene (source of vitamin A) present in excess. While other nutrients like Terpoids, amino acids, flavonoids, steroids, phenols, Coumarins also present. Vitamin C in carrot juices also boosts the immune system. Vitamin A is responsible for young-looking skin, beautiful hair, healthy nails, sharp vision, and strong bones. This paper presents a short knowledge about qualitative as well as quantitative analysis of different components in various kinds of carrots. Moisture content in the red carrot was greater than the orange carrot. PH of both orange and red carrot was neutral.

Conclusion

It is clinched that the withdrawal of beta carotene as of carrots stood efficacious and as well regulate the occurrence of sugars (glucose, sucrose, starch) and moisture content in carrots. To realize the foremost intention of this learning: analysis of diverse gears and extraction and remoteness of beta carotene stood accomplished using diverse practices by using laterally with diverse solvents correspondingly.

Saleem et al.

Soxhlet extraction and decoction extraction was accomplished intended for beta carotene by using varieties of solvent. Quantitative scrutiny for moisture content was reached in this paper by using the AOAC method. Qualitative scrutiny of sugars (glucose, sucrose, starch) proteins, Terpoids, amino acids, flavonoids, steroids, phenols, Coumarins, Glycosides, Anthracyanine, tannins, Quinones, Saponins, PH, the color of extract, taste, was accomplished by using diverse chemicals and devices.

Extract of dissimilar varieties of carrots such as orange, red disclosed positive results for sugars that embrace (glucose, sucrose, starch), protein, Terpoids, amino acids, flavonoids, steroids, phenols, Coumarins. Although Phlobatannins, Saponins, Quinones, tannins were not present in orange carrot. The moisture content in red carrot was 90.3% and in orange carrot was 93.7%. A miscibility chart of beta carotene that realized in this tabloid when beta carotene was miscible in around solvents like acetone, ammonia NH3 solution and immiscibility in solvents like dichloromethane, carbon tetrachloride, chloroform, n-hexane naked that beta carotene is a fat-soluble vitamin which is immiscible in non-polar solvents and carotenoids are water-soluble vitamins both are a font of vitamin A. As a result, in cooperation miscible and immiscible solvents disclosed diverse fallouts.

Acknowledgments

Authors would like to thank Dr. Mussarat Jabeen, Faculty of Chemistry Government Sadiq College Women University, Bahawalpur, Pakistan, for providing necessary facilities, materials and guidance.

Conflict of Interest

Authors have no conflict of interest.

References

Table 1: Extraction of beta carotene from carrots by using different solvents.

<table>
<thead>
<tr>
<th>Sample of Carrot extract</th>
<th>Solvent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red carrots and orange carrots</td>
<td>NH₃</td>
<td>The green color obtained and ppt also formed</td>
</tr>
<tr>
<td>Red carrots and orange carrots</td>
<td>n-hexane</td>
<td>No color change</td>
</tr>
<tr>
<td>Red carrots and orange carrots</td>
<td>Chloroform</td>
<td>No color change layers separate</td>
</tr>
<tr>
<td>Red carrots and orange carrots</td>
<td>Dichloromethane</td>
<td>No color change and layer separate</td>
</tr>
</tbody>
</table>

Table 2: Data and Evaluation

<table>
<thead>
<tr>
<th>Tests</th>
<th>Experiment</th>
<th>Observations</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Took 10ml of extract and poured 2ml of 5% ferric chloride in it.</td>
<td>No color changes.</td>
<td>Tannins were absent.</td>
</tr>
<tr>
<td>Coumarins</td>
<td>Took 1ml of extract and poured 1ml of 10% sodium hydroxide in it.</td>
<td>The yellow color appeared.</td>
<td>Coumarins were present.</td>
</tr>
<tr>
<td>Ninhydrin test</td>
<td>Took 2ml of extract and injected a few drops of 0.2% ninhydrin reagent. Then warmed for 5 minutes.</td>
<td>The blue color appeared.</td>
<td>Amino acids were present.</td>
</tr>
<tr>
<td>Quinones</td>
<td>Took 1ml of extract and poured 1ml of concentrated sulphuric acid.</td>
<td>The brown color changes into a parrot green color.</td>
<td>Quinones was absent.</td>
</tr>
<tr>
<td>Saponins</td>
<td>Took 2ml of extract and 2ml of distilled water were mixed and waggled in a graduated cylinder for 15 minutes lengthwise.</td>
<td>The brownish color appeared.</td>
<td>Saponins were absent.</td>
</tr>
<tr>
<td>Terponids</td>
<td>Took 0.5ml of extract and poured 2ml of chloroform and conc. H₂SO₄</td>
<td>The red-brown color appeared.</td>
<td>Terponids were present.</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>Took 1ml of extract and infused a few drops of 2% HCl in it.</td>
<td>The yellowish color appeared.</td>
<td>Phlobatannins was absent.</td>
</tr>
<tr>
<td>Phenols</td>
<td>Took 1ml of carrot extract and poured 2ml distilled water. Then Injected few drops of 10% ferric chloride in it.</td>
<td>A light green color appeared.</td>
<td>Phenols were present.</td>
</tr>
<tr>
<td>Steroids</td>
<td>Took an equal amount of extract and chloroform. Then added a few drops of concentrated H₂SO₄ in it.</td>
<td>Brown ring and green color appeared.</td>
<td>Steroids were present.</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Took 2ml of extract and poured 2N NaOH in it.</td>
<td>The yellow color appeared.</td>
<td>Flavonoids were present.</td>
</tr>
<tr>
<td>Carbohydrates (Benedict reagent)</td>
<td>Added a small amount of Benedict reagent in carrot extract. Then Heated it in a water bath.</td>
<td>Sky blue color changed into juicy yellow color</td>
<td>Glucose was present.</td>
</tr>
<tr>
<td>Fehling solution</td>
<td>Added a small amount of Fehling solution in carrot extract. Then Heated it in a water bath.</td>
<td>Light green color changed into dark brown color</td>
<td>Glucose was present.</td>
</tr>
<tr>
<td>Sucrose</td>
<td>In a carrot extract added few drops of HCl. Then Heated it for two minutes. Then Added NaOH and benedicts reagent and again heated.</td>
<td>A light green color appeared.</td>
<td>Sucrose was present.</td>
</tr>
<tr>
<td>Starch</td>
<td>In a carrot extract added few drops of iodine solution.</td>
<td>The dark brown color appeared.</td>
<td>Starch was present.</td>
</tr>
<tr>
<td>Biuret test</td>
<td>Took 2ml precipitate solution. Then Added 2ml 10% NaOH in it then added 2-3 drops of copper sulfate. Mixed it gently.</td>
<td>The violet color appeared.</td>
<td>Protein was present.</td>
</tr>
<tr>
<td>Anthracyanine</td>
<td>Took 1ml of extract and poured 1ml of 2N NaOH and warm for 5 minutes at 100°C.</td>
<td>Orange carrot</td>
<td>Orange carrot</td>
</tr>
<tr>
<td>Glycoside</td>
<td>Took 2ml of extract and 3ml of chloroform was poured in it. Ten 10% NH₃ solution was added.</td>
<td>Orange carrot</td>
<td>Orange carrot</td>
</tr>
</tbody>
</table>

Table 3: Different Qualitative test, indicator and results obtained

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Indicator</th>
<th>Result</th>
<th>Food sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Benedicts reagent/ Fehling solution</td>
<td>Positive</td>
<td>Orange and Red Carrot</td>
</tr>
<tr>
<td>Sucrose</td>
<td>HCl, NaOH, Benedict reagent</td>
<td>Positive</td>
<td>Orange and Red Carrot</td>
</tr>
<tr>
<td>Starch</td>
<td>Iodine solution</td>
<td>Positive</td>
<td>Orange and Red Carrot</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>NaOH</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Steroids</td>
<td>Chloroform, conc. H₂SO₄</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric chloride</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Ninhydrin reagent</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Protein</td>
<td>NaOH, CuSO₄</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Coumarins</td>
<td>NaOH</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric Chloride</td>
<td>Negative</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Quinones</td>
<td>Conc. H₂SO₄</td>
<td>Negative</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Saponins</td>
<td>Distilled water</td>
<td>Negative</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Terponids</td>
<td>Chloroform, conc. H₂SO₄</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>HCl</td>
<td>Positive</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Anthracyanine</td>
<td>NaOH</td>
<td>Negative</td>
<td>Orange Carrot</td>
</tr>
<tr>
<td>Glycoside</td>
<td>Chloroform, NH₃ solution</td>
<td>Negative</td>
<td>Orange Carrot</td>
</tr>
</tbody>
</table>

Saleem et al.
International Journal of Natural Medicine and Health Sciences

Original Research

Saleem et al.

Figure 1: Beta carotene extract

Figure 2: Beta carotene and the solvent layer were separated

Figure 3: Qualitative Analysis performed

Figure 4: Beta carotene structure

Figure 5: Powdered beta carotene important applications

<table>
<thead>
<tr>
<th>Minerals (per 100g)</th>
<th>Vitamins (per 100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates 9.15g</td>
<td>C</td>
</tr>
<tr>
<td>Fat 0.20g</td>
<td>B6</td>
</tr>
<tr>
<td>Protein 0.06g</td>
<td>B12</td>
</tr>
<tr>
<td>12 g Magnesium</td>
<td>E</td>
</tr>
<tr>
<td>3.3 mg Calcium</td>
<td>F</td>
</tr>
<tr>
<td>3.1 mg Iron</td>
<td>M</td>
</tr>
<tr>
<td>0.3 mg Zinc</td>
<td>N</td>
</tr>
<tr>
<td>0.163 mg Manganese</td>
<td>P</td>
</tr>
<tr>
<td>0.24 mg Zinc</td>
<td>Zn</td>
</tr>
<tr>
<td>0.69 mg Iron</td>
<td>Fe</td>
</tr>
<tr>
<td>0.69 mg Magnesium</td>
<td>Mg</td>
</tr>
<tr>
<td>1.6 mg Magnesium</td>
<td>Ca</td>
</tr>
<tr>
<td>1.6 mg Magnesium</td>
<td>K</td>
</tr>
<tr>
<td>1.6 mg Magnesium</td>
<td>Na</td>
</tr>
</tbody>
</table>

Figure 6: Amount of different minerals and vitamin present in carrot