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Impact of Thermal, Agitation and Microwave Assisted Extraction Techniques on Ascorbic Acid Content of Red Beet (*Beta vulgaris L*)

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***Abstract:** The processing techniques for food product incorporation or consumption, generally influences the stability of ascorbic acid, same is true for red beet, because ascorbic acid is a natural water-soluble vitamin and highly sensitive to heat, light and air. The objective of the study was to investigate the influence of extraction techniques viz., thermal, agitation assisted and microwave assisted extraction on ascorbic acid content of red beet using different solvent compositions like distil water, distil water with 0.5 % citric acid, 20% ethanol, 20% ethanol with 0.5 % citric acid, 50% ethanol and 50% ethanol with 0.5 % citric acid. The impact of extraction techniques was evaluated on the basis of different temperatures (40, 50 and 60 °C), agitation speeds (150, 170 and 190 rpm) and microwave power levels (160, 320 and 480 W). With Spectrophotometric results it was found that highest ascorbic acid content was in agitation assisted extraction as 104.0132 µg/g dry weight (DW) at 190 rpm in T5 sample (where 50 % ethanol used as solvent) followed by 48.1247 µg/g DW in microwave assisted extraction in T6 (50% ethanol + 0.5% citric acid) and 20.4423 µg /g DW was obtained in thermal extraction at 40°C followed by 20.0757 at 60°C in T5 (where 50 % ethanol used as solvent). The results indicate that solvent type and extraction methods significantly influenced ascorbic acid content extracted in the samples.*
***Keywords:** Red beet, ascorbic acid, thermal, agitation assisted, microwave assisted extraction techniques*

1. Introduction

Red beet (*Beta vulgaris-L)* is a herbaceous biennial or, rarely, perennial plant with leafy stems growing 1–2 meters tall. It comes under the family *Chenopodiaceae* commonly called as table beet, garden beet, red or golden beet, or informally as beet **(Strack *et al.*, 2003).** They are mainly grown for their edible taproots. Three recognized subspecies include *Beta vulgaris-L* subsp. *vulgaris*, *Beta vulgaris-L* subsp. *maritima*, known as the sea beet found throughout the Mediterranean, the Atlantic coast of Europe, and Kashmir and *Beta vulgaris-L* sub.sp*adanensis*, occurs from Greece to Syria **(Azeredo, 2009).** The roots are most commonly deep red-purple in color, but come in a wide variety of other shades, including golden yellow and red-and-white striped.

Ascorbic acid or Vitamin C is a small carbohydrate molecule, also used to prevent and cure scurvy. Scurvy is a pathological life-threatening condition suffered by people who do not have access to fruits or vegetables for long periods of time. Vitamin C, a component of food, necessary for all animals especially humans to prevent scurvy, a disease of the gums, bones and blood vessels and to increase the body's resistance to infection. Ascorbic acid acts as an antioxidant, a nutrient that chemically binds and neutralizes the tissue-damaging effects of substances in the environment known as free radicals. As a result, ascorbic acid is vital for the growth and maintenance of healthy bones, teeth, gums, ligaments and blood vessels. Because of its role in the formation of collagen, the body's major building protein, a central component of all body organs. Therefore, the determination of Vitamin C becomes increasingly important in areas such as Biochemistry, Pharmacology, and Nutrition **(James, 2000).**

Ascorbic acid occurs naturally in many fruits and vegetables, particularly in tomatoes, citrus fruit, cantaloupe, broccoli, spinach, green peppers, cabbage, pineapple, melons, mangoes and potatoes. This vitamin is easily destroyed by cooking or canning foods and by exposure to air and light. A healthy diet generally contains sufficient quantities of ascorbic acid, but the body requires more of the vitamin after serious injury, major surgery, burns and when exposed to extreme temperature. At risk for deficiency are smokers, women taking contraceptives containing the female sex hormone estrogens and people who live in cities with high levels of carbon monoxide from traffic. There is conflicting evidence that taking large doses of ascorbic acid will either prevent the common cold or reduce the severity of its symptoms. Ascorbic acid is an organic compound of carbon, hydrogen and oxygen. Pure ascorbic acid is a white solid and is made synthetically from the sugar dextrose. It is used both in vitamin supplements and as a food preservative.

Ascorbic acid is the Lactose -2,3,-dienol-L-gluconic acid and is an odourless, white solid having the chemical formula $C_6\ H_8O_6$. Vitamin C is the L-enantiomic form of ascorbic acid which also encompasses the oxidation product of dehydroascorbic. It participates in numerous biochemical reactions, suggesting that vitamin C is important for body process from repair **(Rickman *et al*, 2007).** Ascorbic acid is a colourless and odourless crystalline substance, slightly sour in taste and optically active. Only the L-Isomer has antiscorbutic properties. It is soluble in water and alcohol but practically insoluble in chloroform, ether and light petroleum. It has a melting point about 190^{o}C (374^{o}F) (with decomposition), boiling point about 553^{o}C (1027^{o}F), density 1.694 g/cm^3, molar mass 176.12g/mole, pH 3 (5% sol), stable under ordinary conditions. It is readily oxidized, particularly in the presence of Copper and Iron but not of Aluminium. It is for this reason that the foods cooked in copper utensils lose ascorbic acid quickly. This vitamin is also rapidly destroyed by alkalis but is fairly stable in weak acid solutions. Therefore, baking soda has a deleterious effect, but steam cooking destroys very little amount of ascorbic acid. However, freezing has no detrimental effect on this vitamin.

As the extraction process is extremely important for the production of natural colorants, different research groups have put forth great efforts to develop efficient extraction processes **(Santos, *et al.*, 2010)**. Recently, emerging techniques such as sub and supercritical fluid extraction **(Serra *et al.*, 2010; Ghafoor *et al.*, 2010)** and microwave- assisted extraction **(Li *et al.*, 2012; Liazid *et al.*, 2011)** have been employed to extract anthocyanins from different fruits.Microwave-assisted extraction (MAE) is a process utilizing microwave energy to facilitate partition analytes from the Red Beetroot Powder matrix into the solvent. The main advantage of this technique is the reduced extraction time and solvent volume as compared to conventional extraction techniques **(Eskilsson and Bjorklund 2001)**.The aim of the research work is to quantify the ascorbic acid through different extraction methods as thermal, agitation assisted and microwave assisted extraction through different combination of solvents and to analyse the effects of different extraction methods and combination of solvents on ascorbic acid content.

2. Materials and Methods

2.1. Procurement of raw materials: Red beet (Beta vulgaris-L) were procured from local vegetable market of Prayagraj. They were stored at 5±1^{o}C. The experiments were generally performed immediately after procurement.

2.2. Sample preparation: The beetroot was washed with running tap water. After removal of wastes by trimming it was grated manually. Grated beetroot was dried in tray dryer at 60 ^{o}C for 6 to 7 hrs. Then it was ground and sieved with 50 mesh No. sieve. Beetroot powder was packed in LDPE through sealing machine and stored in refrigerator till further processing.

2.3. Different composition of Solvents used for Extraction

The Red Beetroot Powders were extracted with each of the four extraction solvents as; i) pure distilled water ii) distilled water with 0.5% citric acid, iii) 20 % ethanol, iv) 20 % ethanol with 0.5% citric acid, v) 50 % ethanol, vi) 50 % ethanol with 0.5% citric acid. The solvents used in the present study have been extensively tried by many researchers for efficient extraction of phenolic compounds **(Ignat*et al*., 2011).** The extracts were stored in a refrigerator and analysed for Ascorbic acid.

2.4. Extraction methods

Mainly three extraction methods as; i) Thermal Extraction, ii) Agitation Assisted Extraction and, iii) Microwave Assisted Extraction Method. In all three methods above six combinations of solvents were used for extraction.

2.5. Thermal Extraction Method (Jackman et al, (1987)

Extraction was carried out at three different temperatures as 40^o C, 50^o C and 60^o C using Hot Plate, Time of Extraction was 1/2 hour, The average particle size of the powder and solid to solvent ratio (SSR) were maintained at 50 mesh and 1/20.

2.6. Microwave assisted extraction (MAE) method

According to previous study, three different power level were selected as (160 W, 320 W and 480 W) which were computed as a percentage of the total power input of 800 W. The experiments were carried out at room temperature for 5 min. The average particle size of the powder and SSR were maintained at 50 mesh and 1/20. The extracts were centrifuged at 4000 rpm for 1/2 hour **(Sikandar, 2012).**

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2.7. Agitation- assisted extraction (AAE) method

To maximise the natural dye extraction yield by AAE through orbital shaker, the optimum rpm was investigated. Three different agitation speed of 150 rpm, 170 rpm and 190 rpm were selected, and the runs were conducted at 25^{o} C for 1/2 h. The average particle size of the powder and SSR were maintained at 50 mesh and 1/20. The extracts were centrifuged at 4000 rpm for 1/2 hour **(Navare *et al.*, 2011).**

2.8. Estimation of Total Ascorbic Acid Content

Total ascorbic acid was determined by dinitrophenylhydrazine reagent method. Ascorbic acid is an important chemical antioxidant, which is responsible for the non-enzymatic scavenging of superoxide radical and hydrogen peroxide, regeneration of a-tocopherol in chloroplast and in enzymatic scavenging of H_2O_2 in association with peroxidase. Its estimation is based on the formation of pink coloured complex due to the reduction of dinitrophenylhydrazine by ascorbic acid to phenyl hydrazone in acidic medium **(Mukherjee and Choudhuri 1983).** The absorbance is recorded at 530 nm.

2.8.1. Quantification

The concentration of ascorbic acid in the Red Beetroot Powder is calculated from a standard curve plotted by taking known concentration of ascorbic acid (25 - 250 nmol) and following the steps as mentioned for Red Beetroot Powders.

3. Results and Discussion

3.1. *Influence of Temperature during thermal extraction of ascorbic Acid in Beetroot*

Estimation of Ascorbic acid (AA) of red Beetroot obtained by Thermal Assisted Extraction (TAE) through different solvent composition have been recorded as shown in Table 1. and Figure 1. Table depicts that AA vary with temperature and solvent combinations. Maximum AA 20.4423 µg /g dry weight (DW)was obtained at 40^{o}C followed by 20.0757 at 60^{o}C in T_5 (where 50 % ethanol used as solvent) and minimum 13.6861 µg /g DW at 60^{o}C in $T_{2.}$ (where distil water + 0.5 % citric acid was used as solvent).

3.1.1. *Effect of Temperature*

Estimation of Ascorbic acid (AA) of red Beetroot obtained by Thermal Assisted Extraction (TAE) through different solvent composition have been recorded as shown in Table 1. and Figure 1. Table depicts that AA vary with temperature and solvent combinations. Maximum AA i.e. 20.4423 µg /g DW was obtained at 40^{o}C in T5 (where 50 % ethanol used as solvent) and minimum 13.6861 µg /g DW at 60^{o}C in T2. (Where distil water + 0.5 % citric acid was solvent).

It was found that maximum retention of Ascorbic acid was at 40 ^{o}C and minimum was at 60 ^{oC}. It can be concluded that as temperature increased ascorbic acid content was decreased. Similar results were also discussed by **Babalola *et al.*, (2010),** who studied the effect of boiling of green leafy vegetables on ascorbic acid and found that there is about 94.90% loss of vitamin C, when compared with other processing methods. Blanching in hot water can cause an appreciable loss in vitamin C that is thermally labile. Ascorbic acid oxidase needs to

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be inactivated; this prevents enzyme-catalyzed reaction during processing. It can be inferred from results that the lower the temperature, the higher the availability of vitamin C in fruit juice.

Different solvents might account for the different results. Our reports are in agreement with previous work which tells that heat and water reduce vitamin C content, cooking reduce the vitamin C content in fruit juice because vitamin C content is sensitive to heat, water, and air. Ascorbic acid is water soluble vitamin, it is first leached out of the fruit into the water, and then degraded by the heat **(Murcia, *et al.*, 2000)**.

Table1. Influence of Thermal extraction on total ascorbic content from red beet root

	Ascorbic Acid Content (µg/g DW)			
Solvent Code	**40°C**	**50°C**	**60°C**	**Mean±Std. Dev**
T1	15.8043	16.2524	17.2766	16.4444±0.7547
T2	17.7479	16.9158	13.6861	16.1166±2.1456
T3	17.102	18.324	15.9614	17.1291±1.1816
T4	18.9002	16.491	17.4919	17.6277±1.2103
T5	20.4423	20.0233	20.0757	20.1804±0.2283
T6	19.1678	17.6432	19.4821	18.7644±0.9836
Mean±Std. Dev	18.1941±1.6495	17.6083±1.408	17.3289±2.3388	
F-Test	0.004			
Mean±Std. Dev	17.7104±1.7691			

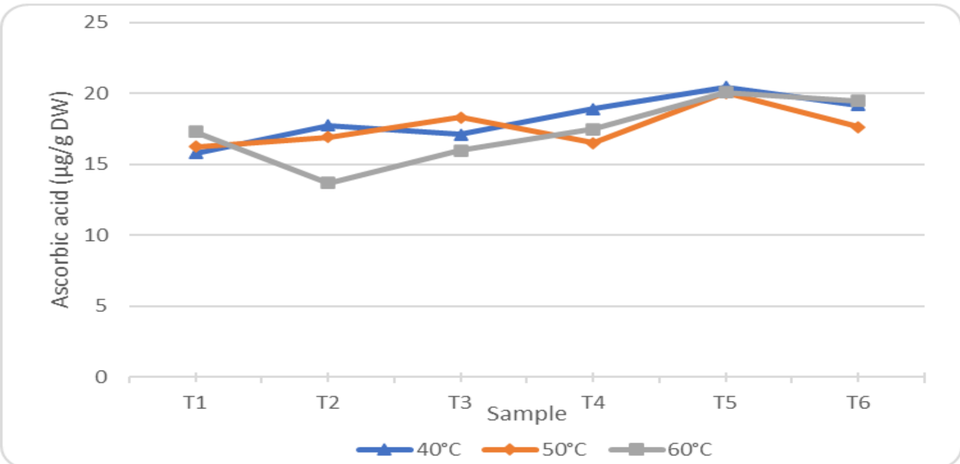

Figure 1. Influence of Thermal extraction on total ascorbic acid from red beet

3.2. *Influence of Agitation Assisted Extraction on Ascorbic Acid from Red Beetroot*

3.2.1. Effect of Agitation Speed

Estimation of Ascorbic acid (AA) of red Beetroot obtained by Agitation Assisted Extraction (AAE) through different solvent composition have been recorded as shown in Table 2. and Figure 2. Table depicts that Ascorbic acid was found minimum i.e. 21.1057 µg/g DW at 150 rpm in sample T_3 (20 % ethanol used as solvent) and maximum 104.0132 µg/g DW at 190 rpm in T_5 sample(where 50 % ethanol used as solvent) followed by 100.1957µg/g DW at 170 rpm in T_5 sample. Ascorbic acid levels could be enhanced by non-conventional methods, such as agitation assisted extraction. The increase in organic acids probably resulted from an increase in organic acid accessibility to the solvent from the disruption of the cell wall by the non-conventional methods. Previous research regarding influence of agitation speed depicts that the higher agitation of magnetic stirring may have caused disruption of cell wall leading to the release of more oxidative and hydrolytic enzymes. These might be attributed to the increment of extraction efficacy caused by cell wall breakage and the release of ascorbic acid from the cells by the non-conventional methods. These observations were consistent with previous results reported by **Luthria and Mukhopadhyay, (2006)** in eggplants, who found that chlorogenic acid levels could be enhanced by non-conventional methods, such as sonication, stirring and rotary shaking

Table 2. Influence of agitation assisted extraction on total ascorbic acid content from red beet

	Ascorbic Content (µg/g DW)			
Solvent Code	**150 RPM**	**170 RPM**	**190 RPM**	**Mean±Std. Dev**
T1	18.487	30.3758	42.317	30.3933±11.915
T2	17.2009	54.4153	42.2705	37.9622±18.9776
T3	16.8809	69.2545	74.7305	53.622±31.9363
T4	21.1057	75.4696	62.9173	53.1642±28.464
T5	21.3675	100.1957	104.0132	75.1922±46.6526
T6	20.5005	84.4371	97.7458	67.5611±41.2954
Mean±Std. Dev	19.2571±1.9942	69.0247±24.3226	70.6657±26.5778	
F-Test	0			
Mean±Std. Dev	52.9825±31.3935			

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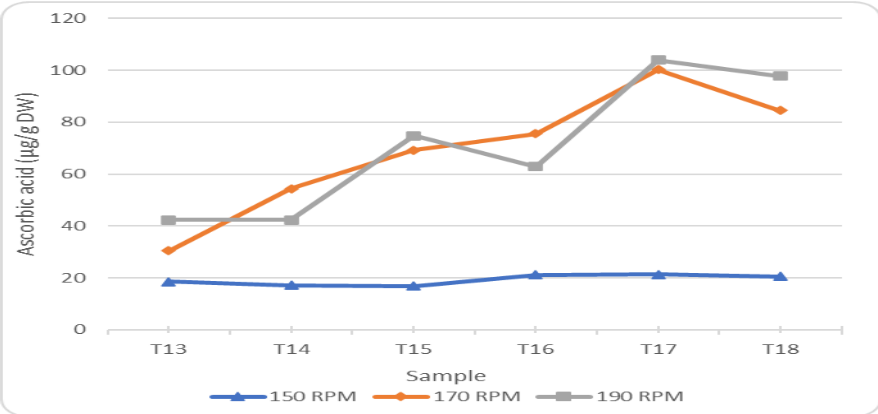

Figure 2. Influence of agitation assisted extraction on total ascorbic content from red beet

3.3. *Effect of Microwave Assisted Extraction on Ascorbic acid of Red beet*

3.3.1. Effect of MW Power level

Estimation of Ascorbic acid (AA) of red Beetroot obtained by Microwave Assisted Extraction (MAE) through different solvent composition have been recorded as shown in Table 3. and Figure 3. Table depicts that, highest ascorbic acid was found at 320 Watts (W) as 48.1247µg/g DW in T_6(50% ethanol + 0.5% citric acid) followed by 42.1773 µg/g DW in T_5 (50% ethanol)and minimum at 11.853 µg/g DW in T_1 (distil water). Increment of microwave power from 160 to 320 W, there is increase in the yield of ascorbic acid. Additional microwave power i.e. 480 W reduced the yield of ascorbic acid due to excessive time and temperature of extraction process destroyed the ascorbic acid**.** Increment of microwave power indicates more heat delivered into beetroot suspension, thus causing more ascorbic acid to be extracted similar results were discussed by (**Nurdjanah and Usmiati 2006**).

Increasing the microwave irradiation energy, the penetration of solvent into the plant matrix can be enhanced and can efficiently deliver to plant cells for ascorbic acid extraction. Molecular interaction with the electromagnetic field offers a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted **(Yan *et al.*,2010)**. As a polar solvent, water can efficiently absorb microwave energy and leads to efficient heating. Moreover, the microwave heating accelerates cell rupture and sudden temperature rise and internal pressure increase inside the cell of plant sample, and in turns the exudation of ascorbic acid within the plant cells into the surrounding solvents and increases the extraction yield. The degradation of cellulosic cell wall at higher temperature when subjected to microwave also increased permeability of solvents into the bio-matrix. This corroborates the earlier reports of **(Zhang *et al.*, 2008).**

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Table 3. Influence of microwave assisted extraction on total ascorbic content from red beet root

	Ascorbic Content (µg/g DW)			
Solvent Code	**160 W**	**320 W**	**480 W**	**Mean±Std. Dev**
T1	20.7856	11.853	19.4995	17.3794±4.829
T2	16.5666	13.4242	16.7237	15.5715±1.8613
T3	14.14	20.8613	21.1755	18.7256±3.9744
T4	16.2582	14.3145	16.4386	15.6704±1.1777
T5	21.0708	42.1773	27.0123	30.0868±10.884
T6	17.4919	48.1247	26.9366	30.8511±15.6871
Mean ± Std. Dev	17.7188±2.7189	25.1258±15.924	21.2977±4.7378	
F-Test	0.045			
Mean± Std. Dev	21.3808±9.6458			

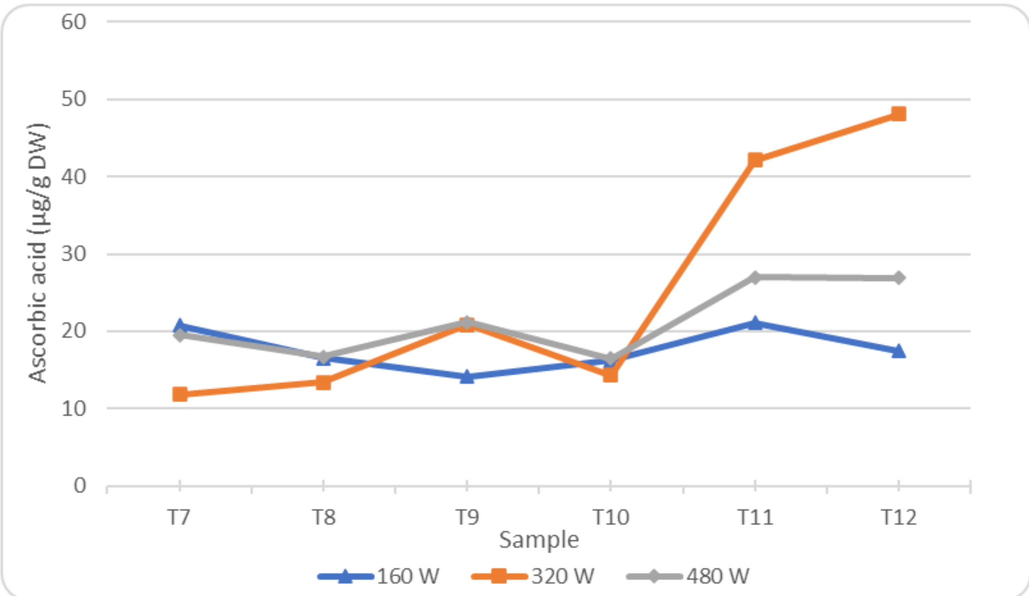

Figure 3. Influence of microwave assisted extraction on total ascorbic acid content from red beet

Conclusion

This study indicated that extraction methods and combination of solvents play significant role in stability of ascorbic acid. Agitation assisted extraction method provides highest ascorbic acid content followed by microwave assisted extraction. Solvent as 50 % ethanol showed highest extraction capacity than others followed by 50 % ethanol and 0.5 % citric acid. Similarly, temperature, agitation speed and microwave power level play significant role in the process of extraction.

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