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Impact of Supercritical Fluid CO_2 (SFE) and Headspace Solid-Phase Micro- Extraction (HS-SPME) on Bioactive Compounds of Gamma-Irradiated *Hibiscus sabdariffa* L. Calyces

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Abstract: This study investigated stable accessions of *Hibiscus sabdariffa* L. subjected to gamma irradiation (0, 200, and 250 Gy) over a four-year cropping period. The research focused on the anthocyanin composition and the efficacy of supercritical fluid carbon dioxide ($SCCO_2$) (SFE) in isolating and enriching bioactive compounds from hibiscus calyces. By utilizing Supercritical Fluid Extraction (SFE) and Headspace-Solid Phase Microextraction (HS-SPME) coupled with Gas chromatography / Mass Spectroscopy (GC/MS), the study demonstrated that $SCCO_2$ is a superior method for capturing volatile oils and thermolabile compounds that are typically lost during traditional isolation. Acetic acid and anethole were identified as the primary volatile constituents in both control and mutant powders. Furthermore, High Performance Liquide Chromatography (HPLC) analysis revealed that before extraction, the 250 Gy mutant exhibited the highest delphinidin levels, while the 200 Gy mutant showed a significant increase in pelargonidin compared to the control.

Key words: Mutation, Gamma irradiation, Headspace-Solid Phase Microextraction, Supercritical Fluid Extraction, GC/MS, HPLC.

1. Introduction

The most prevalent method for creating new plant variants has been radiation-induced mutation. This approach focuses on enhancing current cultivars by modifying specific traits that restrict metabolite yields. While the use of protected germplasms has become increasingly difficult due to rising legal regulations, induced mutations continue to be essential for advancing breeding programs. Notably, medicinal and aromatic species remains largely underutilized in the field of mutation breeding **(Agrawal and Kumar, 2021).**

Hibiscus spp. is among the most important ornamental and medicinal plants, widely valued for their attractive flowers and rich content of bioactive compounds such as phenolics, flavonoids, and anthocyanins **(Ali *et al*., 2021)** and **(Da-Costa-Rocha *et al*., 2014).** These compounds contribute to the plant's antioxidant, therapeutic, and industrial value, making Hibiscus a promising target for genetic improvement **(Mahadevan *et al*., 2009).** However, conventional breeding in *Hibiscus* is often constrained by limited genetic variability, long generation cycles, and reproductive barriers, particularly in vegetatively propagated or highly heterozygous cultivars **(Datta, 2023).** Therefore, alternative breeding approaches are required to accelerate the development of improved cultivars with superior morphological and biochemical traits.

Mutation breeding has emerged as an effective tool for creating novel genetic variability without altering the basic genetic background of elite cultivars **(Ahloowalia *et al*., 2004) ; (Datta, 2023).** Among physical mutagens, gamma rays are widely used due to their high penetration ability, reproducibility, and effectiveness in inducing stable and heritable mutations **(Joint, 2018).** Gamma irradiation can cause structural and physiological changes at the DNA level, leading to variations in plant growth, flowering behavior, branching pattern, and flower coloration **(Kovacs and Keresztes, 2002).** In ornamental and medicinal crops, such induced mutations have been successfully exploited to improve yield-related traits, aesthetic quality, and the accumulation of valuable secondary metabolites **(Maluszynski *et al*., 2001)** and **(Jain, 2010).**

Hibiscus sabdariffa, a tropical member of the *Malvaceae* family, serves as a powerful phytochemical source for managing various metabolic and inflammatory conditions, including hypertension, diabetes, and obesity **(Gurrola-Díaz *et al*., 2010); (Moyano *et al*., 2016)** and **(Riaz et al., 2018)**. These therapeutic properties are attributed to its dense profile of bioactive constituents, notably hibiscus acid, anthocyanins, flavonoids, and phenolic acids.

Anthocyanins have emerged as highly sought-after constituents across the food, beverage, cosmetic, and nutraceutical sectors. These bioactive molecules offer significant health benefits, including enhanced visual acuity, increased antioxidant defense, and management of Type II diabetes. Furthermore, their consumption is linked to a decreased risk of coronary heart disease and the prevention of oncological development **(Ameer *et al*., 2017)** and **(Giuffrida *et al*., 2018)**.

The intense crimson hue of Roselle (*Hibiscus sabdariffa*) calyces is primarily attributed to a specific profile of four anthocyanins. Delphinidin 3-sambubioside (hibiscin) and cyanidin 3-sambubioside serve as the primary pigments, while delphinidin 3-glucoside and cyanidin 3-glucoside are present in smaller quantities **(Wong *et al*., 2002)**. Beyond their chemical makeup, these calyces are most frequently utilized to produce aromatic, deep-red infusions served both hot and chilled. Furthermore, Roselle extracts function as natural colorants in the food industry, appearing in beverages, jams, and jellies where they provide a distinctively tart flavor **(Idham *et al*., 2017).**

A defining feature of supercritical fluid extraction (SFE) is the utilization of solvents in their supercritical state. These fluids facilitate superior extraction rates owing to their high diffusivity and low viscosity, which promote rapid solvent penetration into the solid matrix. Furthermore, SFE offers the unique advantage of tunability, as the fluid's density can be precisely adjusted by manipulating the system's pressure and temperature **(Da Silva *et al*., 2016).**

Carbon dioxide (CO_2) is the most common solvent for Supercritical Fluid Extraction (SFE) due to its safety profile and environmental friendliness. Its low critical temperature of 31.2 °C is particularly advantageous, as it allows for the extraction of heat-sensitive bioactive compounds without causing thermal degradation. Additionally, CO_2 protects samples from oxidation by displacing oxygen, and it is valued for being both highly accessible and recyclable **(Khaw *et al*., 2017).**

The effectiveness of Supercritical Fluid Extraction (SFE) lies in the synergy between pressure and temperature, which directly modifies the solvent's physical characteristics, such as density, viscosity, and diffusivity. This tunability allows for a high degree of selectivity when isolating specific bioactive compounds—a precision unique to the SFE process. Typically, extraction is conducted within a temperature range of 40–60 °C and pressures between 200 and 400 bar **(Da Silva *et al*., 2016)**. Consequently, SFE has become a prominent alternative for the recovery of natural compounds from botanical sources **(Belwal *et al*., 2018), (Ameer *et al*., 2017) and (Giuffrida *et al*., 2018).**

Solid-phase microextraction (SPME) represents a significant "green" milestone in sample preparation for volatile compounds. Over the last twenty years, it has gained widespread use, particularly for profiling the aromatic components of herbal medicines **(de Koning *et al.*, 2009)**. A standard headspace SPME (HS-SPME) procedure coupled with gas chromatography (GC) involves extracting and concentrating volatiles onto a specialized polymeric coating on a silica fiber. Once loaded, the fiber is inserted directly into the GC injection port for thermal desorption **(Srivastava and Singh, 2004).**

Therefore, the present study aims to evaluate the effects of gamma ray irradiation at doses of 200 and 250 Gy on a selected Hibiscus cultivar. The study focuses on key of yield components traits, including plant height, number of fruits / plants, and calyx weight, in addition to assessing changes in active ingredient content. The findings of this research are expected to contribute to the effective application of mutation breeding in *Hibiscus* improvement programs and to support the development of enhanced cultivars with superior ornamental and phytochemical characteristics and the effect of supercritical and SPME on the aroma and bioactive compounds in roselle calyces as well as, investigate the volatile constituent profiles using solid phase microextraction (SPME) coupled to GCMS.

2. Materials and Methods

2.1. Irradiation Treatment

Air dry seeds of (*Hibiscus sabdariffa* L.) variety "Sabahia 17" were irradiated with gamma irradiation at dose of 0, 200, and 250 Gy. This process was conducted using a ^{60}Co Russian Gamma chamber with a dose rate of 665.6 Gy/h, located at the Cyclotron Project within the Nuclear Research Center of the Egyptian Atomic Energy Authority.

2.2. Plant material

Roselle (*Hibiscus sabdariffa* L.) variety "Sabahia 17" used in this investigation. Dry seeds were divided into three groups. The first group was kept without irradiation as control, while the rest were exposed to 200 and 250 Gray gamma-irradiation doses. A field experiments were carried out in the experimental farm of Plant Research Department, Isotopes Application Division, Nuclear Research Center, Atomic Energy Authority, Inshas, Sharkiya Governorate, Egypt, during the four successful seasons of 2022 to 2025. Seeds were planted in newly reclaimed low-fertility sandy soil with low content of fertilizer elements and organic matter on the 10 to 15 th May for every seasons. At harvest, ten seeds were taken randomly from each M_1 plant (M_2 seed). The 10 M_2 seeds from each plant of each bulk were mixed to represent seed of the respective M_2 bulk. These seeds of M_2 bulks were kept for use in experiments of the second season. Individual plant selection was practiced in the M_2 and M_3 season, for high yield/plant. Field evaluation of M_4 selections and the parent were conducted to evaluate selected individual genotypes as compared to the parent. Each treatment was planted in 5 rows, 5 m long and 0.5 m wide, making an area of 17.5 m^2. Hills were 30cm apart; 3 seeds per hill then thinned, three weeks later to one plant/hill. The experimental design used was a randomized complete blocks arrangement in 3 replications. Other agricultural practices such as: irrigation and weeding were carried out as recommended.

2.3. Yield components

In every season seed sowing to harvest (120 - 155 days), number of fruits/ plant and fresh, dry weight of calyx/ plant (g) and plant height (PH) were taken on 25 plants form each replicate.

2.4. Supercritical Fluid CO_2 Extraction Method

To prepare the extract, 250 g of air-dried, finely ground roselle calyces (1 mm particle size) were loaded into a 1 L extraction vessel (Applied Separations, Inc., Allentown, PA, USA). The Supercritical Fluid Extraction (SFE) process, illustrated in Figure 1, began with a 60-minute static phase, followed by a 3-hour dynamic extraction period. Operating conditions were maintained at a supercritical pressure of 300 bar and a temperature of 50 °C. The final yield was determined gravimetrically and reported as a weight percentage (%) based on the mass of the dried extract.

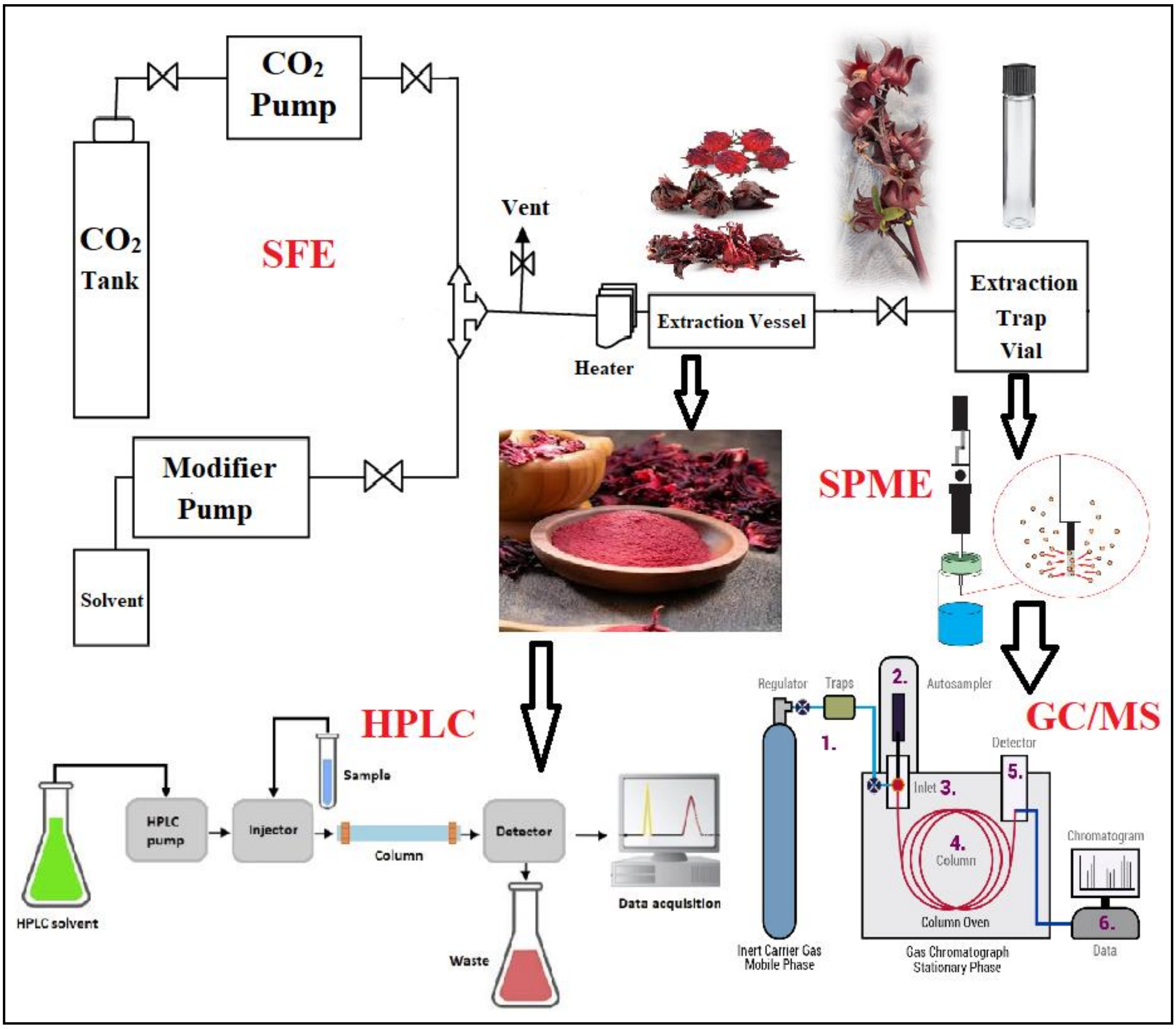


Fig. (1). Schematic diagram of extraction and chromatographic conditions

2.5. Extraction of volatile organic compounds by headspace solid-phase microextraction (HS-SPME)

The Solid-Phase Microextraction (SPME) was conducted using a manual holder equipped with a StableFlex 95/10 µm Carbon-WR-PDMS fiber (Supelco, Bellefonte, PA, USA). For each analysis, 1 g of finely powdered raw propolis was sealed in a 5 mL flat-bottom headspace vial using a magnetic crimp cap and PTFE/silicone septa. To ensure fiber purity, it was reconditioned in the GC injector at 250 °C for 3 min prior to sampling. Extraction was performed by exposing the fiber to the sample headspace for 30 min at a controlled temperature of 60 °C. Following equilibrium, the fiber was immediately transferred to the GC injector for thermal desorption, which was carried out in splitless mode at 250 °C for 1 min. This procedure represents a modified version of the methodology described by **(Pellati *et al.*, 2013).**

2.6. Gas Chromatography–Mass Spectrometry (GC/MS) analysis

Chromatographic analysis was conducted at the Central Laboratory of Chromatography (Radioisotopes Application Division, Nuclear Research Center, Egyptian Atomic Energy Authority) using an Agilent Technologies 8890GC system. The unit was equipped with an automated liquid sampler and coupled to an Agilent 5977B series Mass Selective Detector (MSD). Separation was achieved on a DB-5ms capillary column (60 m x 250 µm x 0.25 µm). The oven temperature program commenced at

50°C (2 min), 5°C/min / 110°C (2 min), and further ramped by 5°C/min to a final temperature of 240°C (5 min). Helium served as the carrier gas at a constant flow rate of 1 mL/min. The temperature of injector was set at 250 °C, transfer line temperature was set as 250 °C and ion source at 250 °C. Injection mode was split ratio 50:1 and solvent delay was 4 min. Mass spectra were acquired in electron impact (EI) mode at 70 eV, covering a scan range of 50–550 amu. Data processing was performed using MSD Mass-Hunter software, with compound identification accomplished by matching mass spectra against the National Institute of Standards and Technology (NIST20) library.

2.7. High Performance Liquide Chromatographic (HPLC) analysis

High-performance liquid chromatography (HPLC) was conducted using an HPLC-LC1620A system equipped with two LC pumps and a UV/Vis detector. The separation was achieved on a C18 column (125 mm x 4.60 mm, 5 µm particle size), and data processing was managed via Chem Station software. Separation was performed under isocratic conditions using a mobile phase consisting of 0.01% formic acid, 22.5% HPLC-grade methanol, and 50% HPLC-grade acetonitrile (v/v/v). Prior to injection, both the mobile phase and samples were filtered and degassed by sonication. The analysis was carried out at a constant flow rate of 1 mL/min with detection at a wavelength of 521 nm **(Durst and Wrolstad, 2001).**

2.8. Statistical Analysis

Data collected across generations were subjected to analysis of variance (ANOVA) using MSTATC statistical software and combined analysis were obtained. Statistical procedures followed the methods described by **(Gomez and Gomez, 1984)**. Then, Duncan's multiple range test **(Duncan, 1955)** was used to verify the significance of mean performances for all traits recorded in both years.

3. Results and Discussion

3.1. Mutation Breeding Program

Mutation breeding using gamma irradiation has proven effective in enhancing key agronomic traits of roselle (*Hibiscus sabdariffa* L.), a valuable crop for its bioactive calyces. Table 1 presents the mean performance of yield components in irradiated (200 and 250 Gy) and non-irradiated genotypes across M_2, M_3, and M_4 generations during 2023 to 2025 summer seasons.

In M_2, increasing dose from 0 to 250 Gy raised mean plant height from 168.0 to 226.0 cm, fruits per plant from 43.0 to 80.3, fresh calyx weight from 83.6 to 107.2 g/plant, and dry calyx weight from 12.9 to 23.1 g/plant, demonstrating a strong positive response of yield traits to irradiation. Similar trends appeared in M_3, where 250 Gy mutants showed 229.3 cm plant height, 85.7 fruits per plant, 108.4 g fresh calyx weight and 23.9 g dry calyx weight versus 171.0 cm, 43.7 fruits, 85.2 g and 13.0 g for the control, confirming that advantageous mutations were transmitted and expressed in subsequent generations. By M_4, performance was further stabilized, with 250 Gy mutants maintaining superiority (228.7 cm height, 86.7 fruits, 113.7 g fresh and 24.3 g dry calyx weight) over both 0 and 200 Gy treatments, indicating successful selection of high-yielding mutant lines **(Kovacs and Keresztes, 2002).** Across M_2–M_4, control plants showed only small year-to-year variation, whereas irradiated treatments, especially 250 Gy, maintained a clear and stable yield advantage, suggesting that induced mutations became fixed and heritable rather than transient stress responses **(Maruthi *et al*., 2023).**

The stepwise improvement from 0 Gy to 200 Gy and then to 250 Gy in all measured traits implies dose-dependent induction of beneficial allelic changes affecting plant vigor, branching and reproductive sink size (fruit and calyx formation). The consistency of trends over three generations supports the effectiveness of gamma mutation breeding in roselle and fits with the broader literature showing that properly chosen doses can enhance yield and secondary metabolite accumulation without compromising overall plant fitness.

The marked increases in plant height and fruit number under 200–250 Gy indicate that gamma irradiation successfully altered growth-regulating loci, leading to more vigorous canopy development and higher reproductive capacity, which translated directly into increased calyx biomass. The strong gains in both fresh and dry calyx weights per plant at 250 Gy suggest that mutation breeding not only increased sink number (fruits) but also sink size and density, which is particularly valuable in roselle where the calyx is the commercial organ for pigment, acid and phytochemical extraction **(Lagoda, 2012)**. Given that this yield improvements were stable from M_2 to M_4, the selected mutant lines represent promising candidates for variety development and for integration with advanced extraction platforms (SFE and HS-SPME) to maximize production of bioactive constituents in industrial roselle chains **(Joint, 2018)**.

Table (1). Effect of gamma irradiation on growth criteria of some yield components and Plant height of irradiated of non-irradiated Roselle genotypes in M_2, M_3 and M_4 generations (Inshas 2023/2025 season)

Generations Doses	Plant height (PH)			No. of fruits / plant			Fresh calyx weight (g/plant)			Dry calyx weight (g/plant)		
	M_2	M_3	M_4	M_2	M_3	M_4	M_2	M_3	M_4	M_2	M_3	M_4
0 Gy	168^c	171.0^c	171.67^c	43.0^e	43.67^e	44.67^e	83.60^c	85.17^c	84.07^c	12.93^c	13.03^c	13.43^c
200 Gy	223^b	223.7^{ab}	225.00^{ab}	53.7^d	59.00^c	60.67^c	93.80^b	94.03^b	92.07^b	17.4^b	17.63^b	18.10^b
250 Gy	226^{ab}	229.33^a	228.67^{ab}	80.3^b	85.67^a	86.67^a	107.2^a	108.4^a	113.7^a	23.1^a	23.90^a	24.30^a
Mean	205.67^a	208.0^a	208.45^a	59.0^b	62.78^a	64.0^a	94.88^a	95.87^a	96.61^a	17.81^a	18.19^a	18.61^a

Values are means of triplicate determinations. Means with different letters in the same column are significantly different at $p < 0.05$.

3.2. GC/MS Identification

This study aimed to characterize the diversity of volatile profiles in two *Hibiscus* mutants using GC-MS analysis **(Ramírez-Rodrigues *et al*., 2011).** A total of 46 volatile constituents were identified across the samples (Table 2). Specifically, 24 compounds were detected in the control group, while the 200 Gy and 250 Gy mutants contained 26 and 13 compounds, respectively. These identified volatiles were classified into six major chemical groups (furans, terpenoids, aldehydes, acids, alcohols, and esters) as well as minor groups such as aliphatic hydrocarbons, phenols/aromatics, ketones, naphthalenes, pyrans, and pyrroles. Acetic acid and anethole were identified as the primary components in both the control and mutant powders. Compared to the control (11.35%), acetic acid levels were measured at 4.92% in the 200 Gy mutant and 7.82% in the 250 Gy mutant. Meanwhile, anethole concentrations in the 250 Gy mutant (14.97%) remained comparable to the control (14.1%), but significantly decreased in the 200 Gy mutant (8.3%).

The volatile profile of the control *Hibiscus* was characterized by a suite of nine aldehydes, several of which were sensitive to higher radiation doses. While 2-heptenal, octanal, 2-octenal, and decanal remained stable at 200 Gy, they were completely degraded or suppressed at 250 Gy. However, the ubiquitous presence of hexanal, benzaldehyde, and nonanal aligns with previous literature **(Chen *et al*., 1998)** and **(Rodríguez-Medina *et al*., 2009)**. Notably, furfural levels nearly doubled in the 250 Gy mutant (2.39%) compared to the control (1.35%), suggesting a dose-dependent effect on carbohydrate degradation or Maillard-related pathways.

Furthermore, trans-cinnamaldehyde completely disappeared at 250 Gy (from 4.13%). D-Limonene actually increased at 200 Gy (10.59%) but was completely degraded by the time it reached 250 Gy. 2-n-Pentylfuran decreased from 2.62% to zero in 200 and 250 Gy, respectively. The last data suggested that, moderate irradiation doses might release or synthesize certain volatiles, while higher doses lead to total degradation. On the contrary, benzaldehyde remained relatively stable across all treatments (8.7% at 0 Gy vs 8.25% at 250 Gy), indicating it is more resistant to radiation-induced changes than other aromatic compounds.

The aromatic profile of *Hibiscus sabdariffa* is further characterized by the presence of limonene, linalool, estragole, and eugenol, as documented in earlier literature **(Gonzalez-Palomares *et al*., 2009); (Rodríguez-Medina *et al*., 2009).**

Table (2). Relative percentage of volatile compounds in *Hibiscus sabdariffa* (control and M_4 selected mutant powder) using SPME-GC-MS

Peak	RT (min)	Compounds	0 Gy	200 Gy	250 Gy
			%	%	%
1	6.817	Acetic acid	4.92	7.82	11.35
2	9.96	Hexanal	1.89	1.84	1.9
3	10.955	Furfural	1.35	1.13	2.39
4	11.003	3-Furfural	1	ND	ND
5	12.869	methoxy-phenyl-Oxime	2.03	3.9	10.33
6	15.017	2-Heptenal	1.18	1.19	ND
7	15.336	Benzaldehyde	8.7	9.14	8.25
8	15.655	1-Octen-3-one	0.43	1.35	ND
9	15.722	1-Octen-3-ol	0.57	ND	ND
10	16.117	2-n-Pentylfuran	2.62	2.62	ND
11	16.288	Ethyl hexanoate	0.61	1.15	ND
12	16.531	Octanal	1.07	1.29	ND
13	16.726	Hexyl acetate	0.68	1.39	ND
14	17.374	p-Cymene	0.53	1.47	ND
15	17.546	D-Limonene	2.99	10.59	ND
16	17.703	Eucalyptol	ND	1.22	ND
17	17.725	cis-2-Menthenol	0.56	ND	ND
18	18.422	2-Octenal	0.91	1.17	ND
19	18.527	γ-Terpinene	ND	1.11	ND
20	19.912	Linalool	1.05	1.03	ND
21	20.074	Nonanal	2.91	3	3.91
22	21.746	(+)-2-Bornanone	1.35	1.21	ND
23	21.827	2-Nonenal	0.67	ND	ND
24	22.079	3-Methoxyanisole	ND	ND	2.29
25	22.07	2-(1,5-Dimethyl-hexyl)-cyclobutanone	0.61	ND	ND
26	22.422	endo-Borneol	1.73	1.93	ND
27	22.989	Estragole	20.17	20.09	18.96
28	23.06	Decanal	1.08	1.19	ND
29	23.341	Verbenone	5.09	5.54	5.12
30	24.065	5-Methyl-2-(1-methylethylidene	0.83	ND	ND
31	24.155	1,5,9,11-Tridecatetraene, 12-methyl-, (E	0.91	ND	ND
32	24.322	.(4Z,6Z,9Z)-1,4,6,9-Nonadecatetraene	0.6	ND	ND
33	24.427	2-Decenal	1.36	ND	ND
34	24.822	trans-Cinnamaldehyde	4.13	ND	ND
35	25.065	cis-Anethole	14.1	8.3	14.97
36	26.346	Eugenol	1.2	ND	ND
37	26.498	2-Undecenal	0.71	ND	ND
38	26.927	Copaene	0.48	ND	ND
39	27.76	Caryophyllene	2.49	3.05	2.63
40	27.97	(E)-β-Farnesene	1.58	ND	ND
41	28.06	Aromandendrene	0.45	ND	ND
42	31.022	α-Bisabolol oxide B	0.56	ND	ND
43	32.27	α-Bisabolol oxide A	2.01	ND	ND
44	33.089	2,2,6-Trimethyl-1-[(1E)-3-methyl	0.57	ND	ND
45	33.446	Pregnanediol	ND	ND	1.98
46	34.694	n-Hexadecanoic acid	1.72	2.95	6.42

ND = Not detected

The volatiles of hibiscus pomace powder after extraction with SFE were illustrated in Table (3). The highest dose (250 Gy), certain aromatic and chemical components saw a notable rise. This might be due to the release of compounds from the complex matrix or irradiation-induced synthesis. However, cis-Anethole increased substantially from 11.65% to 20.17%. This compound is known for its sweet, anise-like aroma and its rise could significantly change the fragrance of the pomace. Benzaldehyde increased from 1.84% to 3.25%, which typically contributes a bitter almond or cherry-like scent. On the opposite, several primary volatiles compounds were reduced as the irradiation dose increased, likely due to oxidation or structural degradation. Some of these compounds; D-Limonene decreased from 9.10% to 5.41%. This is a common trend with irradiation, as terpenes are often sensitive to ionizing radiation. Unlike in the raw powder, acetic acid in the pomace decreased from 9.20% to 6.39% at 250 Gy. As well as, hexanal and (-)-cis-Carane fell from 3.93% to 1.30% and 2.70% to 0.35%. But, estragole remained a dominant component but showed a slight decrease from 14.66% to 13.12%. in addition, supercritical fluid extraction likely removed a large portion of the initial lipid and volatile fractions, leaving a pomace with a different starting profile than the raw powder.

Table (3). Relative percentage of volatile compounds in *Hibiscus sabdariffa* (control and M_4 selected mutant pomace powder) using SPME-GC-MS after extraction by SFE

Peak	RT (min)	Compounds	0 Gy	200 Gy	250 Gy
			%	%	%
1	5.921	Acetic acid	9.2	2.74	6.39
2	9.931	Hexanal	3.93	1	1.3
3	10.922	Furfural	1.12	0.62	1.19
4	12.269	Isoamyl acetate	1.18	0.41	0.68
5	12.698	Methyl N-hydroxybenzenecarboximidoate	0.76	1.28	2.48
6	13.15	Heptanal	0.38	ND	ND
7	14.998	2-Heptenal	1.36	0.77	2.06
8	15.084	Dehydrosabinene	ND	0.23	ND
9	15.317	Benzaldehyde	1.84	1.89	3.25
10	15.636	1-Octen-3-one	0.54	ND	ND
11	15.65	Bicyclo[3.1.0]hexan-2-ol, 5-methyl-, (1α,2β,5α)-	ND	0.75	ND
12	15.869	2-Methyl-2-hepten-6-one	ND	0.27	0.41
13	16.107	(-)-cis-Carane	2.7	0.6	0.35
14	16.279	Ethyl hexanoate	1.5	1	1.45
15	16.522	Octanal	1.18	0.39	0.63
16	16.712	Hexyl acetate	1.68	0.98	1.24
17	17.107	Bicyclo[3.1.0]hexane, 6-isopropylidene-1-methyl-	ND	0.18	0.28
18	17.369	p-Cymene	1.52	0.78	0.86
19	17.538	D-Limonene	9.1	4.11	5.41
20	17.707	Eucalyptol	ND	1.11	0.44
21	17.965	2-Octyn-1-ol	ND	ND	0.27
22	18.031	Phenyl acetaldehyde	ND	0.21	ND
23	18.417	2-Octenal	1.15	0.53	1.29
24	18.526	γ-Terpinene	0.91	0.52	0.63
25	19.565	2-Carene	ND	0.27	ND
26	19.717	α,Para-dimethylstyrene	ND	0.19	ND
27	19.907	Linalool	1.63	2.24	1.66
28	20.069	Nonanal	2.36	1.09	1.42
29	20.498	Phenylethyl Alcohol	ND	0.53	0.24

Table (3) *(continued)*

Peak	RT (min)	Compounds	0 Gy	200 Gy	250 Gy
			%	%	%
30	20.841	7-(1-Methylethylidene)octahydro-1H-cyclopenta[c]oxepin-1-one	ND	0.21	ND
31	21.584	trans-Verbenol	0.35	0.6	0.22
32	21.741	(+)-2-Bornanone	2.5	6.18	2.56
33	21.822	L-camphor	0.73	0.31	0.31
34	21.893	p-Menthan-3-one	ND	ND	0.25
35	22.079	3-Methoxyanisole	ND	ND	0.85
36	22.417	endo-Borneol	3.42	4.64	2.91
37	21.889	Rhodinal	ND	0.34	ND
38	22.031	1,5,5-Trimethylbicyclo[2.2.1]heptan-2-ol	ND	0.18	ND
39	22.284	α-Terpineol	ND	0.28	ND
40	22.546	Isocamphopinone	0.48	0.43	0.43
41	22.593	Terpinen-4-ol	0.36	0.58	0.31
42	22.693	6-(3-Isopropenyl-2-methyl-1-cyclopropen-1-yl)-6-methyl-2-heptanol	ND	0.2	ND
43	22.979	Estragole	14.66	11.97	13.12
44	23.06	Decanal	ND	0.3	0.44
45	23.114	L-Borneol	1.44	0.83	0.5
46	23.336	Verbenone	10.31	15.64	8.99
47	23.541	p-Menth-1-en-9-al	0.32	0.23	ND
48	24.07	Linalyl acetate	0.56	0.41	0.45
49	24.155	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-	0.78	1.27	1.08
50	24.322	Grandlure II	0.86	1.04	0.84
51	24.427	2-Decenal	0.88	0.39	0.85
52	24.479	3-Hydroxypinocamphone	ND	0.18	ND
53	24.746	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	ND	0.44	ND
54	24.822	trans-Cinnamaldehyde	0.31	0.37	0.36
55	25.06	cis-Anethole	11.65	16.32	20.17
56	26.203	Cedrol	0.32	ND	ND
57	26.233	3-Terpinolenone	ND	0.56	ND
58	26.341	Eugenol	ND	0.35	0.25
59	26.498	2-Undecenal	0.49	0.22	0.65
60	26.927	Copaene	ND	0.21	0.27
61	27.76	Caryophyllene	1.48	2	1.43
62	27.97	(E)-β-Farnesene	0.84	1.33	1.97
63	28.336	Humulene	ND	0.31	0.21
64	28.522	α-Curcumene	ND	1.01	0.61
65	28.527	Cubebol	0.59	ND	ND
66	28.708	α-Himachalene	ND	0.7	ND
67	29.174	β-Funebrene	ND	0.69	0.4
68	31.013	aR-Turmerone	ND	0.61	ND
69	31.117	Ageratriol	0.44	ND	ND
70	31.522	Stigmasterol	ND	0.8	ND
71	33.06	Vitamin E	ND	0.18	0.24
72	34.698	n-Hexadecanoic acid	ND	0.78	0.68

ND = Not detected

It is significant that the SFE process yields a greater abundance of hibiscus acid and its associated derivatives than alternative techniques **(Fernández-Arroyo *et al.*, 2011)**. The effectiveness of this method is further evidenced by comparing the GC/MS chemical fingerprints of the initial hibiscus powder with those of the post-extraction pomace, highlighting the compounds successfully sequestered. The analysis of the supercritical fluid CO_2 extract reveals a much more dramatic shift in concentration for certain key compounds compared to the powder or pomace. This extract represents the most concentrated fraction of the hibiscus chemical profile, and irradiation at 250 Gy significantly alters its composition. As shown in Table (4), the extraction by SFE shows a strong trend toward the increasing of small organic acids and furan-related compounds, which are often markers of chemical breakdown. Thus, acetic acid jumped from 22.00% to 35.45%. which is a massive concentration, making it a dominant component of the extract at 250 Gy. Furfural - which is typically formed through the degradation of sugars or carbohydrate-related molecules - increased from 1.54% to 8.02%, suggesting that the irradiation may be targeting these components within the extract. Benzaldehyde increased from 0.41% to 1.42%, mirroring the trend seen in the pomace. 5-Methylfurfural appeared as a new compound at 250 Gy (0.94%), further supporting the "degradation" signature.

On the contrary, the compounds that showed major reductions and degradations after subjected to higher irradiation dose (250 Gy); anisaldehyde decreased from 2.98% to 0.65%, anethole showed a slight decrease from its initial (45.17%) to 43%, estragole decreased from 2.38% to 0.66%, and D-Limonene was completely eliminated (1.39% to ND).

In a comparison with all three GC/MS profiling's of the extracted and non-extracted hibiscus, quantitatively, the acetic acid percentage in the supercritical CO_2 extract exceeded the concentrations found in both the raw material and the residual pomace. This high yield of hibiscus acid derivatives—a notable advantage over other extraction techniques—can be explained by the affinity between the non-polar CO_2 solvent and the non-polar chains of the target compounds. As demonstrated by **(Pimentel-Moral *et al.*, 2019)**, the non-polar nature of SFE solvents is ideal for recovering such less-polar bioactive molecules. Moreover, Furfural levels were relatively stable in the powder and pomace but spiked in the extract. This indicates that the supercritical extraction may have isolated precursors that are highly susceptible to being converted into furfurals under ionizing radiation.

The Supercritical Fluid CO_2 extract is a highly concentrated "essence" that becomes even more refined (or simplified) under irradiation, favoring a high-anethole/low-terpene profile **(Idham *et al.*, 2017)**. The Pomace remains a complex secondary product that retains a significant amount of the aromatic profile even after extraction and irradiation. Finally, the Unextracted Powder shows the most radical chemical transformations (like the spike in Acetic acid and Oximes), as the radiation acts on the full, unrefined biological matrix of the plant. Thus, for the best preservation of original aromatics, lower irradiation doses are preferable, as 200 Gy and above consistently degrade key terpenes like Limonene across all sample types.

HPLC Analysis of supercritical fluid CO_2 Extracts

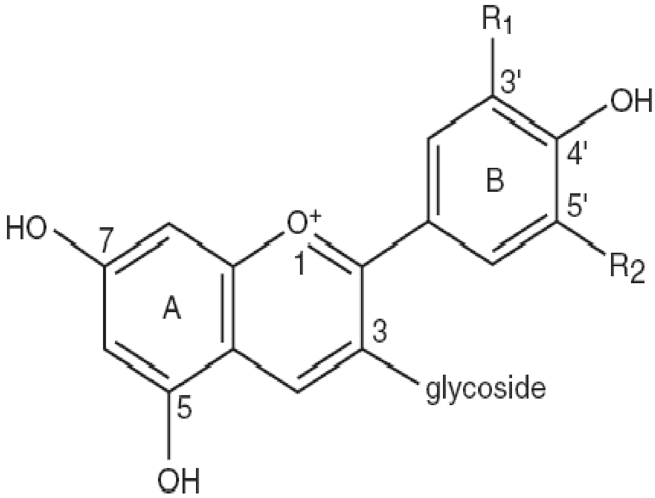
Anthocyanins, a subgroup of the flavonoid family of bioactive compounds **(Elkatory *et al.*, 2023)** are water-soluble pigments responsible for the red, purple, and blue hues found in various fruits, vegetables, and cereal grains. The analysis of these pigments is a vital tool for commodity identification, as each plant source possesses a unique anthocyanin "fingerprint" pattern. Furthermore, HPLC-based anthocyanin profiling is an effective method for verifying the authenticity of fruit juices and other anthocyanin-rich products **(Nagy and Wade, 1995); (Durst and Wrolstad, 2001) and (Gündeşli *et al.*, 2019)**. The potent antioxidant activity observed in *Hibiscus* petals is primarily attributed to its high concentration of polyphenolic compounds, including phenolic acids, flavonoids, and specifically anthocyanins **(Obouayeba *et al.*, 2014); (Prenesti *et al.*, 2007) and (Tavakolifar *et al.*, 2016).**

High-performance liquid chromatography (HPLC) analysis revealed several distinct peaks in the extracts of both control and mutant hibiscus powders (Fig. 2). Among these, four primary anthocyanins were successfully identified: delphinidin-3-O-glycosides (peak 1), pelargonidin-3-O-glycosides (peak 2), cyanidin-3-O-glucoside (peak 3), and peonidin-3-O-glycosides (peak 4).

Table (4). Relative percentage of volatile compounds in *Hibiscus sabdariffa* (control and M_4 selected mutant extract) using SPME-GC-MS after extraction by SFE

			0 Gy	200 Gy	250 Gy
Peak	RT (min)	Compounds	%	%	%
1	6.098	Acetic acid	22	14.14	35.45
2	10.95	Furfural	1.54	1.84	8.02
3	13.475	4-Hydroxybutyric acid	1.04	0.88	1.73
4	15.141	5-Methylfurfural	ND	ND	0.94
5	15.331	Benzaldehyde	0.41	ND	1.42
6	15.668	2-Furyl ethyl ketone	0.87	0.46	1.41
7	16.855	Maple lactone	0.59	0.55	ND
8	17.108	1-Decyne	1.4	0.69	ND
9	17.55	D-Limonene	1.39	ND	ND
10	17.631	Benzyl alcohol	1.53	0.59	0.78
11	17.86	Dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone	1.67	1.07	0.95
12	19.365	2-Furyl hydroxymethyl ketone	3.02	2.83	0.74
13	20.079	trans-2-Undecen-1-ol Formula	0.62	ND	ND
14	20.341	Maltol	1.56	1.37	0.78
15	20.755	2-Methyl-4-heptanone	0.45	0.34	ND
16	21.827	2-Nonenal	0.4	ND	ND
17	21.836	Cyclohexanamine, N-(benzoyloxy)-	ND	0.67	ND
18	22.932	2,5-Dihydrothiophene	0.64	0.99	ND
19	22.984	Estragole	2.38	1.62	0.66
20	23.37	2,4-Nonadienal	0.58	ND	ND
21	23.512	5-Hydroxymethylfurfural	0.63	1.7	ND
22	24.074	Linalyl acetate	0.39	ND	ND
23	24.451	Anisaldehyde	2.98	3.06	0.65
24	25.184	Anethole	45.17	52.25	43
25	26.122	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl	ND	0.44	ND
26	26.365	trans-m-Propenyl guaiacol	ND	0.53	ND
27	26.512	2-Dodecenal	ND	0.34	ND
28	27.189	Vanillin	ND	0.35	ND
29	27.76	Caryophyllene	0.57	1.88	ND
30	27.97	cis-β-Farnesene	0.37	0.43	ND
31	28.07	Aromandendrene	ND	0.86	ND
32	28.303	α-Himachalene	0.88	1.2	ND
33	28.532	Ar-Curcumene	ND	0.7	ND
34	28.715	Longifolene	5.59	6.1	0.72
35	28.922	β-Bisabolene	ND	0.5	ND
36	33.06	trans-Pseudoisoeugenyl 2-methylbutyrate	0.36	0.56	ND

ND = Not detected



R_1=R_2=H	Pelargonidin	**(2)**
R_1=OH, R_2=H	Cyanidin	**(3)**
R_1=OCH_3, R_2=H	Peonidin	**(4)**
R_1=R_2=OH	Delphinidin	**(1)**

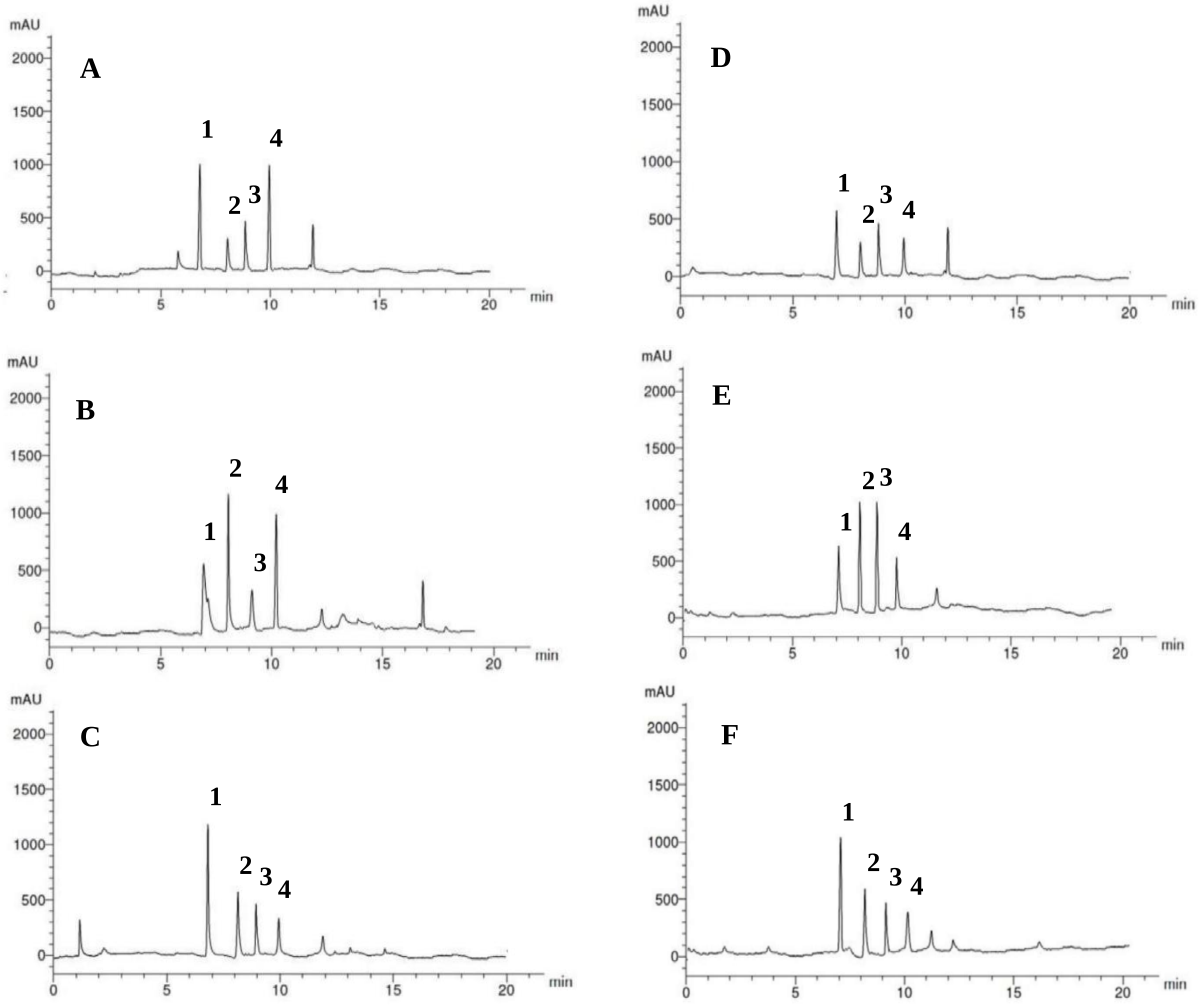


Fig. (2). HPLC profile of some anthocyanins components, before supercritical CO_2 extraction [a) 0 Gy, b) 200 Gy and c) 250 Gy] and after supercritical CO_2 extraction [d) 0 Gy, e) 200 Gy and f) 250 Gy]

As detailed in Table 5 and Fig. 2, the total concentration of anthocyanin components fluctuated significantly both before and after supercritical CO_2 extraction. Furthermore, the anthocyanin composition varied between the control and mutant samples. In the control group, the concentrations of delphinidin-3-O-glycoside, pelargonidin-3-O-glycoside, peonidin-3-O-glycoside, and cyanidin-3-O-glycoside were measured at 15.09, 8.1, 9.4, and 10.2 µg/g, respectively. Following SFE, these levels decreased to 8.7, 5.56, 6.41, and 4.63 µg/g. For comparison, literature indicates that purple shoots of the 'Zixin' cultivar contain approximately 35 µmol/g of total anthocyanins, which is equivalent to 20,860 µg/g of pelargonidin 3,5-di-O-glucoside **(Shen JiaZhi et al., 2018).**

Irradiation treatment caused decreasing in the total content of anthocyanins in the raw (before extraction) samples from 47.93 µg/gm (0 Gy) to 42.50 µg/gm (200 Gy) and finally to 38.42 µg/gm (250 Gy). As well as, cyanidin-3-O-glycosides were the most sensitive to high dose irradiation, dropping sharply from 16.11 µg/gm to 3.55 µg/gm at 250 Gy. Interestingly, certain components increased at specific doses. Pelargonidin - a natural, orange-red flavonoid pigment acting as a powerful antioxidant (Sharma *et al*., 2024) - peaked at 200 Gy (16.07 µg/gm), while Delphinidin - a primary plant pigment responsible for vibrant blue, purple, and magenta colors, and also an antioxidant **(Sharma *et al*., 2024)** - reached its maximum at 250 Gy (19.41 µg/gm), suggesting that irradiation may trigger the conversion of certain precursors into these specific glycosides.

Extraction of hibiscus powder using supercritical CO_2, generally results in a reduction of anthocyanins in the residual pomace, likely due to a combination of partitioning and thermal/pressure degradation during the process. In the non-irradiated hibiscus powder, the total anthocyanin content dropped from 47.93 to 25.30 µg/gm after SFE, representing a retention of approximately 52.8%. while, cyanidin showed the lowest retention after extraction in the control group (only 28.7%), indicating it is particularly unstable during the SFE process.

The combination of irradiation followed by SFE shows that pre-treating the hibiscus with 200 Gy may actually improve the stability or extractability of anthocyanins. where, samples irradiated at 200 Gy maintained the highest total anthocyanin content after SFE (33.69 µg/gm) compared to the 0 Gy and 250 Gy samples. Peonidin-3-O-glycosides exhibited a unique trend; after 200 Gy irradiation, its measurable content in the pomace *increased* relative to its before-extraction value (a retention rate > 100%), which often suggests that irradiation helped release the compound from the plant matrix, making it more detectable after the SFE process. Also, irradiation generally decreases total anthocyanin content in the raw material, a 200 Gy dose appears optimal for preserving or enhancing the recovery of anthocyanins (especially Peonidin and Pelargonidin) during subsequent supercritical fluid CO_2 extraction. High doses (250 Gy) are detrimental to Cyanidin components but appear to favor Delphinidin production.

Table (5). HPLC of anthocyanin components (µg/gm) in hibiscus (control and M_4 selected mutant powder) before and after supercritical CO_2 extraction

	RT (min)	Anthocyanin components (µg/gm)					
		Before SFE Extraction			After SFE Extraction		
		Control	200 Gy	250 Gy	Control	200 Gy	250 Gy
Delphinidin-3-O-glycosides	7.0	15.09	9.12	19.41	8.70	7.25	10.0
Pelargonidin-3-O-glycosides	8.1	7.32	16.07	8.14	5.56	10.2	8.13
Peonidin-3-O-glycosides	9.4	9.41	5.21	7.32	6.41	10.7	6.09
Cyanidin-3-O-glycosides	10.2	16.11	12.1	3.55	4.63	5.54	4.13

4. Conclusion

Based on the results obtained over four consecutive growing seasons, the study concludes that the mutant derived from the 250 Gy gamma irradiation treatment of Hibiscus sabdariffa is the most promising selection. This superiority is primarily attributed to its significantly higher anthocyanin content compared to the other treatments. In addition, mutants derived from both 200 Gy and 250 Gy doses exhibited a marked improvement in their volatile compound profiles relative to the non-irradiated control plants. These enhancements suggest that gamma irradiation is an effective tool for inducing beneficial biochemical variations in Roselle, particularly at higher doses.

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Reference

Agrawal, L. and Kumar, M. (2021). Improvement in ornamental, medicinal, and aromatic plants through induced mutation. Journal of Applied Biology and Biotechnology, 9, 162 - 169. DOI: 10.7324/JABB.2021.9422

Ahloowalia, B.S., Maluszynski, M. and Nichterlein, K. (2004). Global impact of mutation-derived varieties. Euphytica, 135, 187-204. https://doi.org/10.1023/B:EUPH.0000014914.85465.4f

Ali, E. A., Alhasan, A.S., Najeeb, H.F., and Al-Ameri, D. T. (2021). Influence of nitrogen fertilizer on growth and calyx yield of roselle (*Hibiscus sabdariffa* L.) grown under field conditions. In "IOP Conference Series: Earth and Environmental Science", Vol. 735, pp. 012052. IOP Publishing. DOI 10.1088/1755-1315/735/1/012052

Ameer, K., Shahbaz, H.M. and Kwon, J.H. (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review. J Comprehensive reviews in food science food safety, 16, 295-315. https://doi.org/10.1111/1541-4337.12253

Belwal, T., Ezzat, S.M., Rastrelli, L., Bhatt, I.D., Daglia, M., Baldi, A., Devkota, H.P., Orhan, I.E., Patra, J.K. and Das, G. (2018). A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies. Trends in Analytical Chemistry, 100, 82-102. https://doi.org/10.1016/j.trac.2017.12.018

Chen, S.-H., Huang, T.-C., Ho, C.-T. and Tsai, P.-J. (1998). Extraction, analysis, and study on the volatiles in roselle tea. J. Agric. Food Chem., 46, 1101-1105. https://doi.org/10.1021/jf970720y

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I. and Heinrich, M.J. (2014). Hibiscus sabdariffa L.–A phytochemical and pharmacological review., Food Chemistry, 165, 424 - 443. https://doi.org/10.1016/j.foodchem.2014.05.002

Da Silva, R.P.F.F., Rocha-Santos, T.A.P. and Duarte, A.C. (2016). Supercritical fluid extraction of bioactive compounds. Trends in Analytical Chemistry, 76, 40-51. https://doi.org/10.1016/j.trac.2015.11.013

Datta, S. K., (2023). "Induced mutation breeding," Springer. Pp-436. https://doi.org/10.1007/978-981-19-9489-0

de Koning, S., Janssen, H.G. and Brinkman, U.A. (2009). Modern methods of sample preparation for GC analysis. Chromatographia, 69, 33-78. https://doi.org/10.1365/s10337-008-0937-3

Duncan, D. B. (1955). Multiple range and multiple F tests. J of Biometrics, 11, 1-42.

Durst, R. W., and Wrolstad, R.E. (2001). Separation and characterization of anthocyanins by HPLC. Current protocols in food analytical chemistry, F1. 3.1 - F1. 3.13. **https://doi.org/10.1002/0471142913.faf0103s00**

Elkatory, M. R., Hassaan, M.A., Soliman, E.A., Niculescu, V.C., Raboaca, M.S. and El Nemr, A. (2023). Influence of Poly (benzyl oleate-co-maleic anhydride) Pour Point Depressant with Di-Stearyl Amine on Waxy Crude Oil. Polymers (Basel), 15. Pp. 306. https://doi.org/10.3390/polym15020306

Fernández-Arroyo, S., Rodríguez-Medina, I.C., Beltrán-Debón, R., Pasini, F., Joven, J., Micol, V., Segura-Carretero, A. and Fernández-Gutiérrez, A. (2011). Quantification of the polyphenolic fraction and in vitro antioxidant and in vivo anti-hyperlipemic activities of Hibiscus sabdariffa aqueous extract. Food research international, 44, 1490-1495. https://doi.org/10.1016/j.foodres.2011.03.040

Giuffrida, D., Donato, P., Dugo, P. and Mondello, L. (2018). Recent analytical techniques advances in the carotenoids and their derivatives determination in various matrixes. Journal of Agricultural Food Chemistry, 66, 3302 - 3307. https://doi.org/10.1021/acs.jafc.8b00309

Gomez, K.A., and Gomez, A.A. (1984). "Statistical procedures for agricultural research," John wiley and sons.

Gonzalez-Palomares, S., Estarrón-Espinosa, M., Gómez-Leyva, J.F. and Andrade-González, I. (2009). Effect of the temperature on the spray drying of roselle extracts (*Hibiscus sabdariffa* L.). Plant foods for human nutrition, 64, 62-67. https://doi.org/10.1007/s11130-008-0103-y

Gündeşli, M. A., Korkmaz, N. and Okatan, V. (2019). Polyphenol content and antioxidant capacity of berries: A review. International Journal of Agriculture Forestry and Life Sciences, 3, 350-361.

Gurrola-Díaz, C.M., García-López, P.M., Sánchez-Enríquez, S., Troyo-Sanromán, R., Andrade-González, I. and Gómez-Leyva, J.F. (2010). Effects of Hibiscus sabdariffa extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). Phytomedicine, 17, 500-505. https://doi.org/10.1016/j.phymed.2009.10.014

Idham, Z., Nasir, H., Yunus, M., Lee, N., Wong, L., Hassan, H. and Setapar, S.H. (2017). Optimisation of supercritical CO_2 extraction of red colour from roselle (*Hibiscus Sabdariffa* Linn.) calyces. J Chemical Engineering Transactions, 56, 871-876. DOI:10.3303/CET1756146

Jain, S. M. (2010). Mutagenesis in crop improvement under the climate change. Romanian biotechnological letters, 15, 88 - 106.

Joint, FAO (2018). "FAO/IAEA International Symposium on Plant Mutation Breeding and Biotechnology. Book of Abstracts." Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. https://www.iaea.org/sites/default/files/18/08/cn-263-abstracts.pdf

Khaw, K.-Y., Parat, M.-O., Shaw, P.N. and Falconer, J.R. (2017). Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. Molecules, 22, 1186. https://doi.org/10.3390/molecules22071186

Kovacs, E. and Keresztes, A. (2002). Effect of gamma and UV-B/C radiation on plant cells. Micron, 33, 199-210. https://doi.org/10.1016/S0968-4328(01)00012-9

Lagoda, P. (2012). Effects of radiation on living cells and plants. In "Plant mutation breeding and biotechnology", pp. 123-134. CABI Wallingford UK. https://doi.org/10.1079/9781780640853.0123

Mahadevan, N., Shivali, S. and Pradeep, P. K. (2009). Hibiscus sabdariffa Linn. - an overview. Natural Product Radiance, 8, (1): 77 – 83. http://www.niscair.res.in

Maluszynski, M., Szarejko, I., Barriga, P., and Balcerzyk, A. (2001). Heterosis in crop mutant crosses and production of high yielding lines using doubled haploid systems. Euphytica, 120, 387-398. https://doi.org/10.1023/A:1017569617715

Maruthi, R.T., Mangal, V., Kumar, A.A., Meena, J.K. and Mitra, J. (2023). Genetic diversity in advanced breeding lines derived from intraspecific crosses of roselle (*Hibiscus sabdariffa* L.). Vegetos, 36, 675-680. https://doi.org/10.1007/s42535-022-00429-9

Moyano, G., Sáyago-Ayerdi, S.G., Largo, C., Caz, V., Santamaria, M. and Tabernero, M. (2016). Potential use of dietary fibre from Hibiscus sabdariffa and Agave tequilana in obesity management. Journal of Functional Foods, 21, 1-9. https://doi.org/10.1016/j.jff.2015.11.011

Nagy, S., and Wade, R. L. (1995). "Methods to detect adulteration of fruit juice beverages."

Obouayeba, A.P., Djyh, N.B., Diabate, S., Djaman, A.J., N'guessan, J.D., Kone, M. and Kouakou, T.H. (2014). Phytochemical and antioxidant activity of Roselle (*Hibiscus Sabdariffa* L.) petal extracts. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(2): 1453-1465. http://rjpbcs.com/pdf/2014_5(2)/[175].pdf

Pellati, F., Prencipe, F.P. and Benvenuti, S. (2013). Headspace solid-phase microextraction-gas chromatography–mass spectrometry characterization of propolis volatile compounds. Journal of pharmaceutical biomedical analysis, 84, 103-111. https://doi.org/10.1016/j.jpba.2013.05.045

Pimentel-Moral, S., Borrás-Linares, I., Lozano-Sánchez, J., Arráez-Román, D., Martínez-Férez, A. and Segura-Carretero, A. (2019). Supercritical CO_2 extraction of bioactive compounds from Hibiscus sabdariffa. The journal of supercritical fluids, 147, 213-221. https://doi.org/10.1016/j.supflu.2018.11.005

Prenesti, E., Berto, S., Daniele, P.G. and Toso, S. (2007). Antioxidant power quantification of decoction and cold infusions of Hibiscus sabdariffa flowers. Food Chemistry, 100, 433-438. https://doi.org/10.1016/j.foodchem.2005.09.063

Ramírez-Rodrigues, M.M., Balaban, M.O., Marshall, M.R. and Rouseff, R.L. (2011). Hot and cold water infusion aroma profiles of Hibiscus sabdariffa: fresh compared with dried. Journal of food science, 76, C212 - C217. **https://doi.org/10.1111/j.1750-3841.2010.01989.x**

Riaz, G. and Chopra, R. (2018). A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L. Biomedicine Pharmacotherapy, 102, 575-586. https://doi.org/10.1016/j.biopha.2018.03.023

Rodríguez-Medina, I.C., Beltrán-Debón, R., Molina, V.M., Alonso-Villaverde, C., Joven, J., Menéndez, J.A., Segura-Carretero, A. and Fernández-Gutiérrez, A. (2009). Direct characterization of aqueous extract of Hibiscus sabdariffa using HPLC with diode array detection coupled to ESI and ion trap MS. Journal of Separation Science, 32, 3441-3448. **https://doi.org/10.1002/jssc.200900298**

Sharma, P., Kaushik, P., Gupta, S., Malhotra, H. and Panichayupakaranant, P. (2024). "Anthocyanins: Pharmacology and Nutraceutical Importance," Bentham Science Publishers, pp. 224. DOI: 10.2174/97898152238801240100009

Shen JiaZhi, S. J., Zou ZhongWei, Z.Z., Zhang XuZhou, Z.X., Zhou Lin, Z.L. Wang YuHua, W.H., Fang WanPing, F.W., and Zhu Xulun, Z.X. (2018). Metabolic analyses reveal different mechanisms of leaf color change in two purple-leaf tea plant (*Camellia sinensis* L.) cultivars. *Horticulture Research*, 5, 1 – 14. https://doi.org/10.1038/s41438-017-0010-1

Srivastava, S.K. and Singh, S.V. (2004). Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. Carcinogenesis, 25, 1701 - 1709. https://doi.org/10.1093/carcin/bgh179

Tavakolifar, F., Givianrad, M.H. and Saber-Tehrani, M. (2016). Extraction of anthocyanins from hibiscus sabdariffa and assessment of its antioxidant properties in extra virgin olive oil. Fresenius Environmental Bulletin, 25, 3709-3713.

Wong, P.-K., Yusof, S., Ghazali, H. M. and Che Man, Y. B. (2002). Physico-chemical characteristics of roselle (*Hibiscus sabdariffa* L.). Nutrition and Food Science, 32, 68-73. https://doi.org/10.1108/00346650210416994