Impact of radiation processing on some phytochemical constituents of selected Indian vegetables and their products

By

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DECLARATION

I, hereby declare that the investigation presented in the thesis has been carried out by me. The work is original and has not been submitted earlier as a whole or in part for a degree / diploma at this or any other Institution / University.

Jyoti Tripathi

Dedicated to "The Almighty"

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Jyoti

CONTENTS

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SYNOPSIS

Vegetables are vital components of a healthy diet and provide essential nutrients and bioactive phytochemicals. There are evidences that diets rich in vegetables can decrease the levels of chronic diseases including cancer [1, 2]. Vegetables possess high antioxidant properties that are imparted by bioactive compounds. These compounds present in them protect bio-molecules from oxidative damage, thus playing a major health protective role.

In recent years there has been an increase in demand for convenience foods such as ready-to-cook (RTC) vegetables by consumers. Low shelf life of majority of these products, however, restricts their marketability. Apart from microbial safety, maintenance of fresh-like characteristics is the main criteria determining consumer acceptability of the product. Various post-harvest processing techniques are currently available that can kill pathogens while retaining fresh attributes of the produce.

Radiation processing is a promising technology for improving the shelf life of fresh-cut produce. Treatment of food products by ionizing radiation is a physical process involving direct exposure to electromagnetic γ-rays, X-rays or electron beam for improvement in food safety and shelf life. It is a non-thermal technology that effectively eliminates food-borne pathogens in various foods, including fresh vegetables without compromising the nutritional properties or sensory qualities of food [3] Gamma radiation being ionizing in nature causes radiolysis of water thereby producing reactive hydroxyl radical. These radicals are extremely reactive and attack and damage cellular components, especially DNA. Due to damage to genetic material there is inhibition in

microbial growth, thus inactivating microorganisms [4]. Unlike typically processed foods, fresh-cut products consist of living tissues and post harvest processing treatments including irradiation can act as stress bringing about change in post harvest physiology of the product. The critical factors affecting consumer acceptability include microbial and sensory quality (color, texture, flavor /aroma). Apart from the sensory attributes, the bioactive constituents such as phenolic constituents, carotenoids etc. are mainly responsible for the beneficial effects of vegetables. Existing literature suggests an increased extractability and bioavailability of bioactive constituents as a result of radiation processing [5]. An enhanced breakdown of bound precursors including aroma and phenolic glycosides has also been reported. It can therefore be inferred that radiation processing can enhance aroma by hydrolyzing aroma glycosides, increase total phenolics and thus antioxidant capacity, while bringing about microbial decontamination of food products. Ash gourd (*Benincasa hispida*), drumstick (*Moringa oleifera*) and pumpkin (*Cucurbita pepo*) are traditional Indian vegetables used in Indian cuisine. These vegetables have recently been marketed as RTC products due to the convenience they offer. No reports exist so far on the impact of radiation processing on the above vegetables or their RTC products. The present thesis aims at developing radiation processed RTC products of the above vegetables with improved shelf-life and understanding changes in bioactive compounds and their precursors during such a treatment.

Chapter 1 of the thesis introduces the subject of food irradiation with special emphasis on irradiation of minimally processed fresh-cut vegetables and describes the scientific

literature related to the present work. Based on the review of available literature, it was found that radiation processing leads to microbial decontamination thereby improving the shelf-life of fresh-cut fruits and vegetables. It can also lead to increased contents of various bioactive principles, mainly responsible for the beneficial effects of vegetables. However, very few reports have dealt with the impact of radiation processing on the postharvest quality of vegetables of Indian origin. The chapter provides general information on vegetables with special reference to ash gourd, drumstick and pumpkin, the three most widely used Indian vegetables. The current information on the chemical aspects of these vegetables and general methods of isolation and identification of chemical constituents, in particular, the bioactive constituents are detailed. Commonly used methods for isolation and identification of aroma constituents of vegetables and their quantification using instrumental methods such as GC/MS, HPLC are also discussed.

Chapter 2 of the thesis describes the materials and experimental methods. The vegetable samples of ash gourd, drumstick and pumpkin were obtained from local growers in and around Mumbai, India. Irradiation was carried out using a food package irradiator (GC 5000, Board of Radiation and Isotope Technology, India) at BARC, Mumbai.

Gamma irradiation was used for shelf-life extension of RTC vegetables. Sensory quality was assessed by a sensory panel through hedonic testing. Color was evaluated by colorimeter and texture through texture analyzer according to the standard protocols. Nutritional parameters like vitamin content, total phenolic content and antioxidant properties were studied according to standard AOAC protocols. Activities of different

enzymes were assayed as per reported spectrophotometric methods. The non-volatile constituents that included phenolic acids, carotenoids and triterpenes were studied by TLC and HPLC analysis.

Likens-Nikersons simultaneous distillation extraction (SDE) apparatus and solid phase microextraction (SPME) techniques were used for isolation of free aroma volatiles. Aroma glycosides were extracted using XAD and solid phase extraction (SPE) using C-18 reverse phase cartridges. The total ion current obtained from SPME (for free aroma) and SPE (for bound aroma) was further processed using chemometrics (PCA) for analyzing the effect of radiation treatment on aroma constituents.

Chapter 3 deals with the results and discussion. It has been divided into following subsections.

3.1. Development of radiation processed shelf-stable RTC products: Preliminary screening of vegetables was done based on the feasibility of gamma irradiation for shelf-life improvement. Effect of radiation processing (0.5-2.0 kGy) on the shelf life of five RTC vegetables, namely ash gourd (*Benincasa hispida*), pumpkin (*Cucurbita pepo*), bottle gourd (*Lagenaria siceraria*), lady finger (*Abelmoschus esculentus*) and drumstick (*Moringa oleifera*) were studied when stored at 10 °C. In the case of ready-to-cook (RTC) ash gourd, pumpkin and drumstick, radiation processed samples were acceptable up to storage duration of 10-25 d depending on the vegetable and radiation dose delivered. Cut bottle gourd pieces developed browning/ blackening after a few minutes of cutting making them unacceptable. In case of ladies finger, the treated and untreated samples were acceptable only up to a storage period of 1-2 d, beyond which increased

sliminess and off odor in the radiation treated samples restricted their acceptance. Ash gourd, drumstick and pumpkin were therefore selected for the development of radiation processed shelf-stable products. The data obtained for microbial analysis, color, texture and sensory acceptability (hedonic analysis) were separately fitted in cubic polynomial equations for each vegetable. Optimum radiation dose and storage period for each product was determined by solving the model equations using criteria (total mesophilic counts < 5 log CFU/g; overall sensory acceptability > 5 with minimum change in color and texture). For ash gourd, optimum solution was a shelf life of 12 d at a radiation dose of 2 kGy, while for drumstick and pumpkin, the optimum parameters were a shelf life of 12 and 21 d respectively at a radiation dose of 1 kGy.

3.2. Characterization of the developed products: The radiation processed products were characterized for various physical and chemical parameters important for consumer acceptability. Sensory analysis by quantitative descriptive analysis (QDA) indicated that radiation processed products possessed excellent sensory quality at the end of intended storage period. An appreciable increase in DPPH radical scavenging activity was observed as a result of radiation treatment in all the three vegetables which was linearly correlated with increase in total phenolic and flavonoid contents.

Vegetables impart characteristic flavor (aroma and taste) to the cuisine. In the green form, aroma of majority of the vegetables is indistinguishable from each other. Cooking results in liberation of their characteristic aroma from their precursors. Very few reports however exist on the nature of these precursors in vegetables, particularly of Indian origin. The free and glycosidically bound aroma compounds of ash gourd, drumstick and

pumpkin were studied. Despite of the presence of several volatile aroma compounds in a food matrix, not all of them are responsible for the characteristic odor of a food product. Therefore there is a great interest in determining the contribution of each constituent towards the overall flavor of a product. Key odorants of each vegetable were therefore characterized. GC-O analysis of the aroma extracts was successfully employed for the identification of key odorants in the vegetables studied. Acetoin, octanal and nonanal in ash gourd; benzothiazole, decanal and 2E-decenal in drumstick and a combination of 6Z-nonenal and 2E, 6Z-nonadienal in case of pumpkin were identified as the key odorants responsible for the characteristic aroma. Further, the effect of radiation processing and storage on aroma composition was examined. The GC/MS data obtained for free and bound aroma was subjected to Principal Component Analysis (PCA) for each vegetable separately. PCA is an unsupervised technique which is used for dimensionality reduction of multivariate data sets and allows visualization of complicated data for easy interpretation [6]. To know the nature of the constituents responsible for the differences among control and radiation processed samples of different days, factor loading data was analyzed. Contents of alcohols increased in response to radiation processing and storage in ash gourd and pumpkin which could be attributed to the radiolytic breakdown of their corresponding glycosidic precursors. Contents of major carbonyl compounds decreased with radiation processing and storage in ash gourd, while a significant increase in the content of hexanal and trans-2-hexenal was observed in radiation processed drumstick. The increase in aldehyde contents could be attributed to radiation induced lipid radiolysis resulting in release of linolenic acid and its subsequent conversion to

aldehydes via lipoxygenase (LOX) pathway. On the other hand, conversion of aldehydes to corresponding branched chain alcohols and subsequently to esters could be the reason for decreased contents of aldehydes in ash gourd.

A considerable difference in the effect of radiation processing on the content of glycosidic precursors was observed among the three vegetables studied. Two types of radiation effects were observed. Decrease in the content of these precursors was noted in ash gourd and pumpkin, while the contents increased in irradiated drumstick. Both radiation induced increased extractability and degradation was observed. It can be concluded that both these changes can occur simultaneously and independent of each other. Despite the changes in the content of some of the aroma compounds during storage and radiation processing as noted instrumentally and statistically, they were not sufficient to be observed by the sensory panel. Hence the radiation processed product developed, had good sensory acceptability at the end of storage period.

Gamma-radiation (2 kGy) induced inhibition of browning in RTC ash gourd stored (10°C) up to 12 d was further investigated. In the control samples, phenylalanine ammonia lyase (PAL) activity increased during storage that could be linearly correlated with enhanced quinone formation and browning. Radiation treatment resulted in a significant increase in the content of alpha resorcylic acid, a known PPO inhibitor. The decreased PPO activity was thus correlated with the increased content of this acid in irradiated samples. The kinetic parameters of α-resorcylic acid inhibition were determined using Linweaver-Burk plots. The nature of inhibition was found to be mixed and reversible type. No significant change was observed in peroxidase activity. So

browning inhibition in radiation processed (2 kGy) RTC ash gourd during storage could be a synergistic effect of decreased quinone formation and enzyme (PPO and PAL) activities.

3.3. Isolation, identification of bioactive phytochemicals and determining their changes during radiation processing

3.3.1. Ash gourd

3.3.1.1. Plant growth promoting activity: The ash gourd waste (peel and seeds) has been traditionally used as a green manure for increasing the soil nutrients while shelled seeds are reported to have anabolic properties that promote tissue growth. The vegetable extract was therefore screened for the bioactive principles responsible for the plant growth promoting activity. Ash gourd juice was fractionated with solvents of different polarities (ether, ethyl acetate and n-butanol) and the butanol fraction was analyzed by TLC and HPLC. The major compound in this fraction was purified by preparative TLC and identified as acetoin glucoside based on NMR and mass spectral studies as well as by chemical synthesis. In vitro treatment by soaking tobacco leaf discs in MS liquid medium containing different concentrations of this compound in the range of 0.044-0.88 mg resulted in a significant increase in the diameter of leaf discs. At an optimum concentration of 0.176 mg, the diameter of leaf discs increased to 1.13 cm compared to 0.6 cm in control. Two dimensional gel electrophoresis of the proteins isolated from the untreated and acetoin glucoside treated tobacco leaf discs revealed the expression of a new protein in the treated samples. This was identified as a Ras related nuclear GTPase or Ran protein, a very important regulatory protein, by peptide mass finger printing. The

study demonstrated for the first time the role of acetoin glucoside as the compound responsible for the tissue growth regulating properties of the vegetable. The content of acetoin glucoside, the active principle, remained unaffected in radiation-treated ash gourd. Thus the plant growth activity was maintained in the irradiated samples.

3.3.1.2. Angiotensin converting enzyme (ACE) inhibition activity: The juice of ash gourd is recommended to patients suffering from heart ailments and high blood pressure. The ash gourd juice was fractionated with solvents of different polarity (ether, ethyl acetate and water) and each of them was screened for ACE inhibition activity by an enzymatic assay using hippuryl-histidine-leucyl as substrate. The aq extract obtained from non-irradiated control and irradiated ash gourd at a concentration of 50 mg/mL exhibited 47 % and 41 % ACE inhibition respectively. This extract was purified by RP-HPLC and further by gel filtration. The molecular weight of the active fraction as determined by GPC was 323 Da. The purified fraction showed a UV absorbance at 220 and 254 nm. Mass spectral analysis showed the presence of amino acids *viz.* alanine (M+, 89) and valine (M+, 117) in this fraction. This confirms the presence of a small peptide comprising of these amino acids as the active principle. However, the compound requires further characterization which is under progress. The ACE inhibition activity of the ash gourd however remained unaffected due to irradiation.

3.3.2. Triterpenes in pumpkin: Cucurbita glycosides from pumpkin were isolated by extracting with methanol and further purified by preparative TLC. The isolated compounds were subjected to HPLC, where the peak at retention time 4.27 was tentatively identified as cucurbitacin E glucoside. The total extract was enzymatically

hydrolyzed as well as acetylated and then subjected to GC/MS analysis. The mass fragmentation of the identified peaks was compared with that of cucurbitacins reported in literature. Based on this data, two major compounds were identified as Cucurbitacin C and E. Thus based on the HPLC analysis of total extract and GC/MS analysis of enzymatically hydrolyzed and acetylated extract the major triterpenes identified were glycosides of Cucurbitacin C and E. Estimation of the active principles in control and irradiated pumpkin by densitometer indicated a significant ($p < 0.05$) decrease in their contents during storage. Increase in activity of hydrolytic enzymes such as glycosidase and pectinase during storage in fruits and vegetables has been reported [5]. This could account for the decrease in contents of these compounds during storage. However, the contents of identified active principles were unaffected due to radiation processing.

3.3.3. Glucosinolates in drumstick: Effect of radiation treatment (1 kGy) on bioactive glucosinolates in drumstick was investigated. Food processing methods result in their hydrolysis and various breakdown products are formed which are generally identified using GC/MS. Among breakdown products, isothiocyanates (ITCs) have the highest biological activity. They have been reported to possess broad-spectrum antimicrobial activity against bacterial, fungal pathogens and insects [7, 8] and possess potent anticarcinogenic activity. Major isothiocyanates identified in drumstick were isopropyl isothiocyanate (4.38 ng/g), 2-butyl isothiocyanate (1.99 ng/g) and isobutyl isothiocyanate (2.35 ng/g). The content of all the three isothiocyanates increased in response to radiation processing (1 kGy) with a 2-3 times higher content in radiation-treated samples at the end of storage as compared to the respective non-irradiated

samples. The observation suggests an improved nutraceutical value of the vegetable by radiation processing.

3.3.4. Isolation, identification and quantification of phenolic constituents and carotenoids: The phenolic constituents of all the three vegetables were extracted with methanol and subjected to HPLC-DAD analysis. Syringic, chlorogenic and α-resorcylic acid were identified as major phenolic constituents in ash gourd; protocatechuic, p-hydroxybenzoic, vanillic acid and quercetin in drumstick while pumpkin was characterized with the presence of gallic, caffeic, o-coumaric acid and kaempferol. Detailed analysis revealed that radiation processing resulted in increased extraction of phenolic constituents in ash gourd, while no significant effect of radiation processing was observed on the phenolic constituents of drumstick and pumpkin.

Pumpkin is a good source of carotenoid pigments. Apart from imparting color, they act as antioxidants and enhancers of the immune response. The carotenoids of pumpkin were extracted in hexane and analyzed by HPLC-DAD. The major carotenoids identified were lutein and β-carotene. The contents of both the constituents remained unaffected in response to the radiation treatment. To the best of our knowledge, this study demonstrates for the first time the effect of radiation processing on shelf life improvement and on the phytochemical constituents of RTC ash gourd, drumstick and pumpkin.

Finally the achievements of these studies will be highlighted in the conclusion section that will follow chapter 3.

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CHAPTER 1

INTRODUCTION

1.1. Importance of vegetables in the diet

Vegetables form an important part of our daily diet. They provide nutrients vital for health and maintenance of our body. Most vegetables are low in fat and calories with none having cholesterol. They are important sources of both macro nutrients such as fiber, carbohydrates, and micro-nutrients like potassium, folate (folic acid), vitamin A, and vitamin C. Many of the colored vegetables, in particular, dark-green leafy, cruciferous and deep-yellow-orange vegetables, are rich in vitamin C, carotenoids, folates and a range of bioactive phytonutrients. A balance of the micro- and macro-constituents, however, is more likely to be responsible for their health benefits than any single compound. Several biologically active phytochemicals have been identified in food plants. Table 1 lists vegetables that are rich source of specific compounds. However, apart from a few exceptions, these compounds are also present in varying amounts in most other fruits and vegetables.

In 1990, The World Health Organization (WHO) recommended a goal of at least 400 g of vegetables and fruits daily (in addition to potatoes) including at least 30 g of legumes, nuts and seeds [1]. This report, together with other reports from expert bodies, has been translated into a recommendation for the consumption of at least five portions of fruits and vegetables per day. The World Cancer Research Fund and American Institute for Cancer Research have recommended that diets should be based primarily on foods of plant origin, provided that such diets are nutritionally adequate and varied [2].

Table 1. Richest vegetable sources of specific compounds

S.No.	Substance	Richest source
1	Vitamin C	Citrus (and other) fruits, green vegetables, potatoes
2	Vitamin E	Vegetable oils, avocado
3	Folates	Green leafy vegetables, potatoes, oranges
4	Vitamin K	Green leafy vegetables
5	Calcium, iron, magnesium	Green vegetables
6	Alpha and beta-carotene	Carrots, green leafy vegetables
7	Potassium	Vegetables and fruits generally
8	Lutein	Yellow/green vegetables
9	Lycopene	Tomatoes
10	Flavonoids	Onions, apples, green beans
11	Glucosinolates	Brassicas
12	Fibre, NSP, pectin	Fruits and vegetables generally

There are evidences that diets rich in vegetables can decrease the levels of chronic diseases. Extensive reports on the preventive effects of vegetables and fruits against cancer are available in literature [3, 4]. In addition, new scientific evidence is emerging supporting a protective role for vegetables in prevention of cardio-vascular diseases (CVD), cataract formation, age-related macular degeneration, chronic obstructive pulmonary disease, digestive disorders, and possibly hypertension. There may be several biologically plausible reasons why the consumption of vegetables might slow, or prevent,

the onset of chronic diseases. Some of the phytochemical constituents present have capacity to modify antioxidant pathways, detoxification enzymes, the immune system, cholesterol and steroid hormone concentrations and blood pressure by acting as antioxidant, antiviral and antibacterial agents.

Antioxidant effects of vegetables have been of recent interest. Dietary antioxidants have been demonstrated to play a major health protective role. The huge literature data currently available have shown that fresh fruits and vegetables possess high antioxidant properties that are beneficial to human health [5, 6]. The oxidative damage to bio-molecules is held responsible for CVD, cancer initiation, cataract formation, inflammatory disease and several neurological disorders. The antioxidants are recognized as bioactive compounds that protect bio-molecules from oxidative damage. Several trace elements, such as manganese, copper, zinc, iron and selenium, are essential constituents of the antioxidant metallo-enzymes: superoxide dismutase, glutathione peroxidase and catalase. Vitamins C, E and the carotenoids & polyphenols, have received most attention with respect to their antioxidant capability. They can interrupt free radical initiated chain reactions of oxidation, or scavenge free radicals before they damage cellular components. The *in vivo* antioxidant effects of different groups of compounds, have been studied, but their metabolism is complex and effects in *in vivo* may be different both in extent and mode of action from those observed *in vitro* model systems. Indeed, the health-promoting effects of many phytochemicals are attributed mainly to their antioxidant activity, although there could also be other modes. In a nutshell, vegetables are an important part

of our diet, which provide many beneficial nutrients, and protect against several chronic diseases, apart from adding a specific flavor to the cuisine.

1.2. Minimal processing of vegetables

In the past few years, worldwide demand for convenience foods such as minimally processed fresh cut fruits and vegetables has increased considerably. Fresh-cut products are highly popular in Europe and in recent years have gained acceptance in Asia as well. Changing lifestyles and eating habits as well as preference for fresh, healthy and natural is a strong marketing drive for such products. Processed Ready-to-eat (RTE) fresh fruits or ready-to-cook (RTC) vegetables provide convenience without greatly changing their fresh like properties and human health benefits, with a minimal time of preparation before consumption [7].

According to "The International Fresh-Cut Produce Association", fresh-cut produce has been defined as **trimmed, peeled, washed, and cut into 100% usable product that is subsequently bagged or prepackaged to offer consumers high nutrition, convenience, and value while still maintaining freshness** [8].

The USDA and FDA defined "fresh" and "minimally- processed" fruits and vegetables as: **fresh-cut (pre-cut) products which have been freshly-cut, washed, packaged and maintained with refrigeration. Fresh-cut products are in a raw state and even though processed (physically altered from the original form), they remain in a fresh state, ready to eat or cook, without thermal processing, or treatments with additives or preservatives [8].**

Commercial production of RTC vegetables include washing, sorting according to size, peeling, cutting, packaging and finally storage at refrigerated temperatures. However, these products have a very short shelf life. For most fresh-cut produce, shelf-life is best defined as the period within which the product retains acceptable quality for sale to the consumer. It is therefore necessary to identify what 'acceptable quality' means before it can be decided at what point the product no longer satisfies those expectations. From the quality standpoint, it is desirable to preserve the characteristics of fresh-cut vegetables at their peak. The most appealing attributes of these products include their fresh-like appearance, taste and flavor, in addition to convenience without the use of preservatives [9]. Fresh-cut processing increases respiration rates and causes major tissue disruption as enzymes and substrates, normally sequestered within the vacuole, become mixed with other cytoplasmic and nucleic substrates and enzymes. Processing also increases wound-induced ethylene, water activity, and surface area per unit volume, which may accelerate water loss and enhance microbial growth since sugars also become readily available [10]. These physiological changes may be accompanied by flavor loss, cut surface discoloration, color loss, decay, increased rate of vitamin loss, rapid softening, shrinkage, and a shorter storage life. Increased water activity and mixing of intracellular and intercellular enzymes and substrates may also contribute to flavor and texture loss during and after processing. Therefore, proper temperature management during product preparation and refrigeration throughout distribution and marketing are essential for maintenance of quality.

The critical factors for deciding the shelf-life of a fresh-cut produce can be classified into the following categories: microbial spoilage, appearance and color, texture, flavor (taste and aroma) and nutritional value.

1.2.1. Microbial spoilage

During processing of minimally processed vegetables such as peeling, cutting and shredding, the surface of the produce is exposed to contamination with bacteria, yeasts and moulds. Besides, the plant cellular fluids released during processing favor the growth of micro-organisms by providing a nutritive medium for growth. Processing also increases water activity and surface area per unit volume which may accelerate water loss and enhance microbial growth [10]. Most of the minimally processed vegetables have low acid range pH (5.8–6.0), high humidity and a large surface area which can provide ideal conditions for the growth of microorganisms [11]. Thus, fresh cut vegetables have high levels of microorganisms and several outbreaks of food-borne illnesses have been found to be associated with these products [12]. In general, total counts of microbial populations on minimally processed vegetables after processing range from 3.0 to 6.0 log cfu/g [13]. A number of micro-organisms have been found in fresh-cut products including mesophilic microflora, lactic acid bacteria, coliforms, fecal coliforms, yeasts, and pectinolytic microflora [13]. Some of these micro-organisms produce pectinolytic enzymes which degrade the cell structure and as such provide more nutrients for microbiological activity.

Physiological ageing of commodities could also increase microbial counts [14]. Moulds are less important in minimally processed vegetables due to the intrinsic properties such

as slightly acid to neutral pH favoring bacteria and yeasts which generally overgrow moulds [15]. Microorganisms impact the economic value of fresh-cut products by decreasing product shelf-life, through spoilage, and by posing a risk to public health by causing foodborne disease [13].

Mechanical wounding of vegetables enhances a diverse array of enzymatic pathways, associated in many cases with generation of volatiles [16]. Increased concentration of ethanol, acetaldehyde and some aliphatic alcohols such as 2-methyl-1-butanol, 3-methyl-1-butanol, propanol has been reported to be produced by micro-organisms during storage in various fresh-cut produce [17]. However, research on changes in volatile and non-volatile compounds in minimally processed vegetables during storage in relation to microbiological activity is scarce.

1.2.2. Appearance and color

Appearance is determined by physical factors including the size, the shape, the wholeness, the presence of defects (blemishes, bruises, spots etc.), finish or gloss, and consistency. Fruit or vegetable gloss are related to the ability of a surface to reflect light and freshly harvested products are often more glossy [18]. Fresh-cut vegetable products must appear to be fresh, generally indicated by the brightness of color and the absence of visual defects or drip.

Color is derived from natural pigments present in fruits and vegetables. The primary pigments include fat soluble chlorophylls (green) and carotenoids (yellow, orange and red) and the water soluble anthocyanins (red, blue), flavonoids (yellow), and betalins (red). Bright colors of fresh produce attract the buyers. However, color that is not

appropriate for the item, indicative of loss of freshness or lack of ripeness, can decrease the consumer acceptance for the product. For example, white blush in cut carrots is a quality defect [19]. Yellowing in green vegetables due to loss of chlorophyll is unacceptable [20]. Wilting, browning, dull colors, and drip are all indicators of loss of freshness in fresh-cut vegetables [21].

Browning is a serious quality defect in fresh-cut produce. Enzymatic and non-enzymatic reactions may result in the formation of brown, gray, and black colored pigments. The enzymes involved in browning reactions include phenylalanine ammonia lyase (PAL), a key enzyme in the phenolic biosynthesis and polyphenol oxidase (PPO) as well as peroxidase (POD), which catalyse the oxidation of polyphenolic compounds. In intact plant cells, phenolic compounds in cell vacuoles are spatially apart from the oxidizing enzymes present in the cytoplasm. Once tissues are damaged by processing such as peeling and cutting, the mixing of the enzymes and phenolic compounds as well as the easy oxygen diffusion to the inner tissues result in a browning reaction. Further, as a result of minimal processing, phenolic content increases via wound-induced enhancement in PAL (PAL; EC 4.3.1.5) expression. Oxidation of the phenols thus formed to quinones catalyzed by PPO (PPO; EC 1.10.3.1) and peroxidase (POD; EC 1.11.1.7) and subsequent polymerization of the quinones to relatively insoluble brown polymers (melanins) results in unacceptable product [22]. It also leads to off flavors and losses in nutritional quality. For example, russet (brown) spotting and brown stain [23] are undesirable visual defects in lettuce. The main reactions as catalyzed by PPO are shown below (Figure 1). It has been proposed that increase in PAL activity could be used as a predictive index of shelf

life [24]. An increased PAL activity has also been correlated with a decrease in shelf-life and overall visual quality of minimally processed lettuce [24]. Increased enzymatic activities have been reported in fresh-cut potato strips [25], broccoli florets (*Brassica oleracea* var. italica; [26], and lettuce leaf segments [27]. Such visual defects decrease the consumer acceptability and therefore marketability of the fresh-cut produce.

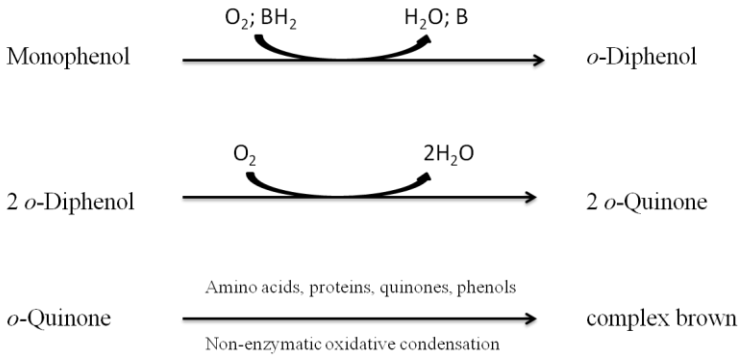


Figure 1. Reactions depicting mechanism of PPO action in browning development.

1.2.3. Texture

According to Bourne [28], the textural properties of a food are the "group of physical characteristics that arise from the structural elements of the food, sensed by the feeling of touch, are related to the deformation, disintegration and flow of the food under a force, and measured objectively by functions of mass, time, and distance". Textural parameters of fruits and vegetables are perceived with the sense of touch, either when the product is picked up by hand or chewed. Consumers have clear expectations regarding the texture of fresh-cut vegetables. Salad vegetables like lettuce, carrot, celery, and radish should be crisp. Undesirable textural attributes are the opposite of the desirable ones. For example, wilted lettuce, limp carrots or celery, and flaccid radish are unacceptable.

Texture is derived from turgor pressure, and the composition of individual cell walls. Cell walls are composed of cellulose, hemicelluloses, pectic substances, proteins, and also lignins in the case of vegetables. In processed fruits and vegetables, changes in texture are strongly related to transformations in cell wall polymers due to enzymatic and non-enzymatic reactions. Cellulose and hemicellulose show minimal changes in structure and composition in most plant based foods [29]. Most of the changes observed in plant based foods are ascribed to transformations in pectin structure and composition. These changes are strongly influenced by the processing steps and conditions. Apart from mechanical injury imposed by processing operations, microbial growth also bring textural changes in minimally processed vegetables during storage [13]. The rapid texture breakdown observed in cut vegetables during storage is often the result of higher aerobic psychotrophic counts. Different micro-organisms produce pectinolytic enzymes including pectate lyase, polygalacturonase and pectin methyl esterases resulting in textural changes. The most commonly isolated pectinolytic bacterial species are *Erwinia* and *Pseudomonas*. Pectinolytic yeasts and moulds include *Trichosporon sp* and *Mucor sp,* respectively [13].

While generally flavor is being cited as the most important quality attribute, textural defects and the interaction of flavor and texture are more likely to cause rejection of a fresh product [30]. Consumer and taste panel responses indicate that individuals are actually more sensitive to small differences in texture than flavor [8], making texture a crucial parameter for acceptability.

1.2.4. Flavor (taste and aroma)

An overall flavor of food results from the combined effect of its various constituents on the human olfactory organs. Taste sensation is perceived once the food product is taken in the mouth, while smell (olfaction) can be sensed by the odor of an object at a distance.

1.2.4.1. Taste

Taste can be classified into five basic categories - sweetness, sourness, saltiness, bitterness and umami. In Asian countries within the sphere of mainly Chinese and Indian cultural influence, pungency (piquancy or hotness) had traditionally been considered a sixth basic taste. Sweetness, usually regarded as a pleasurable sensation, is produced by the presence of sugars, some proteins, and a few other substances. It is often connected to aldehydes and ketones, which contain a carbonyl group. Sourness in the taste is detected by the acidity content of the food product. The sourness of substances is rated relative to dilute hydrochloric acid, which has a sourness index of 1. Citric acid, malic acid and oxalic acid are the acidic compounds attributing sourness to fruits and vegetables. Many of the fruits are naturally sour in taste such as lemon, grape, orange, and tamarind. Saltiness is a taste produced primarily by the presence of sodium ions. The saltiness of substances is rated relative to sodium chloride (NaCl), which has an index of 1 [31]. Bitterness is an undesirable taste found in some fresh-cut vegetables such as salad greens [32] and vegetables of cruciferae family. When Cruciferae cells are ruptured, glucosinolates undergo enzymatic hydrolysis with the endogenous myrosinase enzymes, releasing thiocyanates, isothiocyanates [33], sulphate, and glucose [34]. These breakdown products cause bitter taste. Umami is an appetitive taste and is described as a savory [36]

or meaty [36]. It can be tasted in cheese and soy sauce, and while also found in many other fermented and aged foods, this taste is also present in tomatoes, grains, and beans[36]. The amino acid, glutamic acid is responsible for umami taste [37] but some nucleotides (inosinic acid and guanylic acid [37] can act as complements, enhancing the taste. Processing and packaging precautions must be taken to ensure that off-odors and off-flavors do not jeopardize the marketability of the fresh-cut vegetables.

1.2.4.2. Aroma constituents

Although taste sensations are very important, it is the presence of trace amounts of (usually) many volatile compounds which are crucial in determining the flavor quality of a food product.

The aroma of vegetables is generally released during processing such as cutting and cooking. It is generated due to the presence of a large number of organic volatile compounds which are present in extremely small concentrations. Two largest groups that contribute to natural odors are the aliphatics derived mainly from fatty acids and terpenoids synthesized by the maevalonate or the methylerythritol pathway. Among the aliphatic compounds derived from fatty acids with carbon chains between two to seventeen, the C6 compounds such as (Z)-3-hexenyl acetate, hexenol, hexenal and hexanol belong to a well known group of green-leaf volatiles (GLVs) found in several fruits and vegetables. Terpenes constitute the largest family of natural plant products including fruits and vegetables. They are made up of homologous series of repetitive five carbon isoprene units in their structure. These include the monoterpenes (C10, 2 isoprene units), sesquiterpenes (C15, 3 isoprene units), diterpenes (C20, 4 isoprene units),

triterpenes (C30, 6 isoprene units), tetraterpenes (C40, 8 isoprene units) and polyterpenes ([C5]n, where n may be 9-30,000). Among these, the monoterpenes and sesquiterpenes are the major constituents of several essential oils derived from plants and plant products. Terpenes can be further sub divided into terpene hydrocarbons and oxygenated terpenes depending on the nature of their functional groups. Some of the monoterpene hydrocarbons such as myrcene, α-pinene and sabinene are widely distributed in fruits and vegetables and have pleasant and characteristic odor. Oxygenated terpenes commonly exist as alcohols, aldehydes, ketones and esters.

Other odorant chemical classes include the benzenoids and phenylpropanoids derived via the phenylpropanoid pathway, lactones derived from hydroxyl fatty acids and C-5 branched chain compounds derived from branched-chain fatty acids. Among the nitrogen containing compounds, amines, oximes and indoles are the most common. Sulfur containing compounds such as hydrogen sulfide, methanethiol, dimethyl sulfide and isothiocyanates are derived from amino acid metabolism. Figure 2 represents some representative examples of aroma compounds existing in the nature.

In vegetables, the volatiles representing their characteristic flavor are generally esters, aldehydes, alcohols, terpenes or their derivatives. Sometimes, a single compound alone can approximate the flavor of a product and thus is termed as “impact compound”. However, in other cases a combination of several constituents that, together, interact with the receptors from the nasal mucosa creates a sensory impression in the brain typical of the product [38].

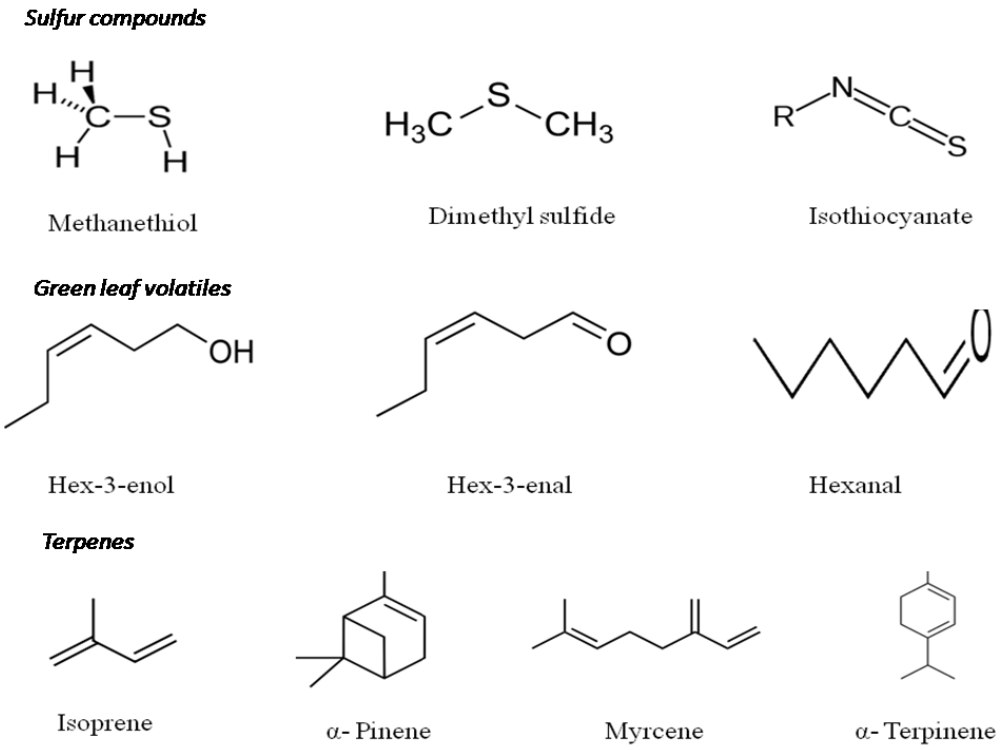


Figure 2. Representative aroma compounds of vegetables

Besides the volatile constituents that contribute to odor, there exists a class of non-volatile glycosidically bound odor precursors that are widely distributed in the plant kingdom [39]. Although odorless, they are able to release free aroma by enzymatic hydrolysis during processing such as cutting and cooking and hence are considered to be potential aroma compounds that play a crucial role in the overall food quality. Some of the bound volatile compounds have unique odor properties that provide the characteristic flavor to a food product. The aglycone bound to the glycoside moiety may include both terpenoid and non-terpenoid structures. Mono and sesquiterpenoids, aliphatic alcohols, alkyl phenols and norisoprenoids are the most prominent. Generally the sugar which is directly bound to the aglycone is β-D-glucose. This glucose moiety may or may not be further substituted with additional sugar units. The second sugar unit is reported to be either α-L-

arabinofuranose, α-L-rhamnopyranose, β-D-xylopyranose, β-D-apiofuranose, or β-D -glucose (Figure 3). Most of the hydrolases employed for release of volatile aglycones are exo-glycosidases. Initial step in the cleavage of disaccharidic conjugate therefore require the action of α-L-arabinosidase, α-rhamnosidase, β-D-xylosidase, or β-D-apiosidase, for the cleavage of the intermediate sugar linkage. A second hydrolytic step further liberates the aglycone moiety by β-glucosidase activity [40]. Only in the case of gentiobiosides (β-D-glucopyranosyl-(1-6)- β-D-glucopyranosides), where the disaccharidic sugar consists of two glucose units, β-D-glucosidase activity is able to liberate the aglycone in a two-step mechanism [41].

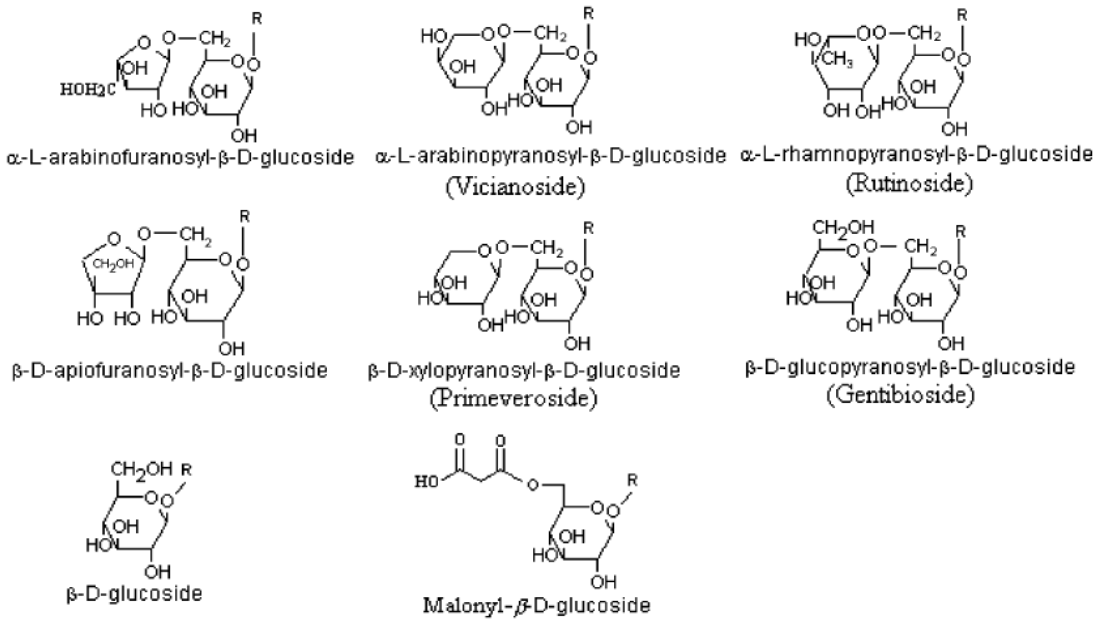


Figure 3. Structrue of glycosidic portion of aroma glycosides

The intact fresh vegetables are nearly odorless and are indistinguishable from each other. However, processing such as cutting and cooking, results in liberation of characteristic

aroma from their precursors. Very few reports however exist on the nature of free as well as bound aroma compounds in vegetables, especially in Indian vegetables. Further, reports on the effect of various processing on aroma of vegetables and their products are lacking. Therefore, it is of interest to study the volatile profile of vegetables and to identify the role of these compounds in providing characteristic aroma to these products.

1.2.5. Nutritional value

The beneficial effects of vegetables have been attributed to non-essential food constituents, which are known as phytochemicals or bioactive compounds. These phytochemicals are considered to be biologically active secondary metabolites that in many cases provide color and flavor, and are commonly referred to as phytoprotectants or nutraceuticals [42]. Plant derived phytochemicals have been shown to be associated with many health-promoting effects such as protection against inflammation, cardiovascular diseases, diabetes, asthma and cancer [43]. Fresh vegetables are good sources of dietary fiber, minerals, vitamins, and other beneficial phytochemicals. The major classes of phytochemicals important in vegetables include carotenoids, phenolics, and glucosinolates.

1.2.5.1. Carotenoids

Fruits and vegetables contain different amounts and types of carotenoid [44]. Chemically carotenoids are polyisoprenoid compounds and can be classified into two main groups: (a) carotenes composed only of carbon and hydrogen atoms and (b) oxygenated derivatives, xanthophylls, that contain at least one oxygen function such as hydroxy, keto, epoxy, methoxy or carboxylic acid groups. Their structural characteristic is a conjugated

double bond system which influences their chemical, biochemical and physical properties. This class of natural pigments occurs widely in nature. They are responsible for the attractive colors of many flowers, fruits and vegetables [44]. This attribute is of great importance in foods, since color is often a criterion of quality and is typically modified by food processing [45]. Carotenoid content in fruits and vegetables depends on several factors such as genetic variety, maturity, postharvest storage, processing and preparation.

The physiological role of these compounds has resulted in a great interest in their biological function [46]. In addition to the provitamin A activity of some carotenoids, they also have other functions, such as antioxidants and enhancers of the immune response. Furthermore, some of them are involved in the cell communication. Xanthophylls have also been shown to be effective as free radical scavengers [47]. Figure 4 shows some representative examples of the carotenoids found in fruits and vegetables.

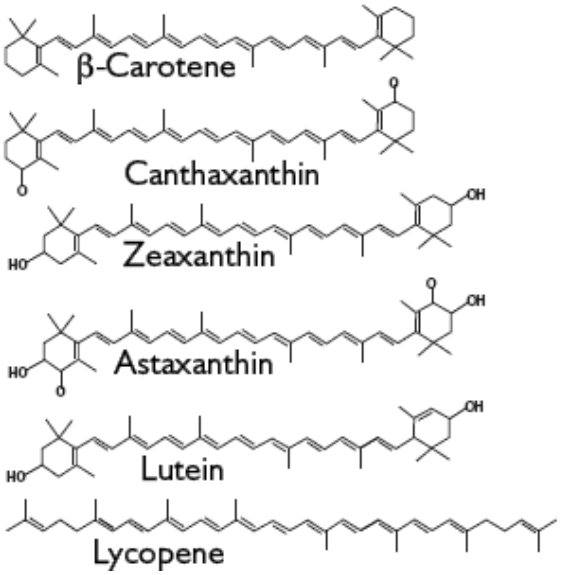


Figure 4. Some representative carotenoids found in vegetables.

1.2.5.2. Phenolics

Phenolic compounds refer to the main classes of secondary metabolites in plants. Chemically, phenols are cyclic benzene compounds possessing one or more hydroxyl groups associated directly with the ring structure. Phenolic compounds are important in deciding overall quality since they contribute to organoleptic characteristics such as colour, astringency, bitterness, and aroma. Table 2 shows the organoleptic notes associated with certain phenolic compounds. Phenolics are produced in plants as secondary metabolites via the shikimic acid pathway [48]. Phenylalanine ammonialyase (PAL) is the key enzyme catalyzing the biosynthesis of phenolics from the aromatic amino acid, phenylalanine [48]. They can be classified based on the number and arrangement of the carbon atoms as flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones and others) and nonflavonoids (phenolic acids, hydroxycinnamates, stilbenes and others). They are commonly found conjugated to sugars and organic acids.

1.2.5.2.1. Flavonoids

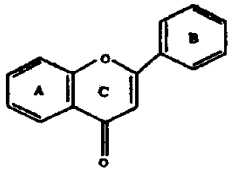
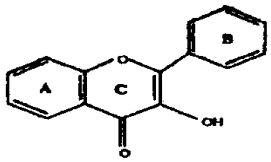
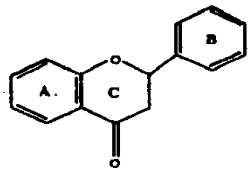
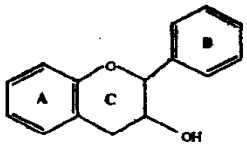
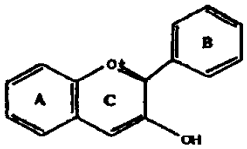
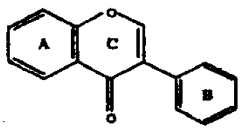
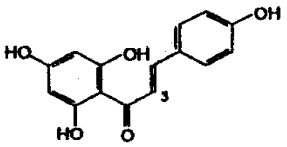
Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge (C6-C3-C6). They are the most abundant phenolic compounds found throughout the plant kingdom. The individual flavonoid subgroups are shown in Table 3. Flavonols are the most widespread of the flavonoids. Kampferol, quercetin, isorhamnetin, myricetin and their O-glycosides are some of the flavonols reported in vegetables. In fresh vegetables, generally flavonols exist only as glycosidic precursors or acylated by various hydroxycinnamic acids [49]. Within the

flavonoids, anthocyanins are the most important group of water-soluble colored plant pigments, possessing antioxidant activity and other useful biological properties. They are generally found in the form of glycosides, with aglycones being rarely found. They are involved in protecting the plants against excessive light and also have an important role in attracting pollinating insects. The stability, color intensity and potential biological activity of anthocyanins is determined by their chemical structure.

Table 2. Sensory descriptors reported for simple phenols

Compound	Description
4-Vinlyphenol	Strong, smoky aroma
2-Methoxy-4-vinylphenol	Pleasant, clove-like aroma
m-Cresol	Smoky aroma
p-Cresol	Phenol-like aroma
p-Ethylphenol	Powerful woody-phenolic aroma
Isoeugenol	Carnation aroma
Isoeugenyl acetate	Weak rose-carnation aroma, initially burning then sweet taste
Isoeugenyl benzyl ether	Faint rose-carnation aroma
Isoeugenyl ethyl ether	Aroma similar to isoeugenol
Isoeugenyl formate	Faint orris-like, green, sweet, woody aroma; warm, spicy flavor
Isoeugenyl methyl ether	Delicate, clove-carnation aroma; burning, bitter taste
Isoeugenol phenylacetate	Intensely sweet, carnation, vanilla, clove-like, green, woody aroma; slightly fruity flavor
2-Methoxy-4-methylphenol	Sweet, spicy vanilla-like aroma; bitter taste
Phenol	Smoky aroma
4-Ethylguaiacol	Soy sauce flavor
Guaiacol	Sweet aroma, burnt aroma, smoky taste
4-Methylguaiacol	Smoky aroma, smoky taste
2,6-Dimethoxyphenol	Woody, medicinal aroma, smoky aroma, smoky taste

Table 3. The subgroups of flavonoids (Table adapted from Karakaya, S. (2004) [50])

Main structure	Flavonoids	Chemical structure	Samples
$C_6C_3C_6$	Flavones	 FLAVONES	Cinencetin, nobiletin, tangeritin, isocinencitin, luteolin, apigenin
	Flavonols	 FLAVONOLS	Quercetin, kaempferol
	Flavanones	 FLAVANONES	Hesperidin, naringenin
	Flavanols (catechins)	 CATECHINS	(+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin
	Anthocyanins	 ANTHOCYANIDINS	Peonidin, delfinidin, petunidin, cyanidin
	Isoflavones	 ISOFLAVONES	Daidzein, genistein
	Chalcones	 Chalcones	Phloretin, arbutin, chalconaringenin

1.2.5.2.2. Phenolic acids

Phenolic acids are aromatic secondary plant metabolites, widely spread throughout the plant kingdom. Hydroxycinnamic acids such as ferulic, sinapic, caffeic, and p-coumaric, are among the most widely distributed in plants. Table 4 shows the phenolic acids isolated from various vegetables [51]. They occur in most tissues in a variety of conjugated forms. Esters and amides are the most frequently reported types of conjugates, while glycosides rarely occur. The most abundant member of dietary hydroxycinnamates are ferulic and caffeic acids [52]. Most vegetables contain caffeic, ferulic, sinapic or coumaric acid conjugated with quinic acid and/or esterified with sugars [53].

Table 4. Phenolic acids isolated from vegetables.

Vegetable	Compound
Broccoli	p-Coumaric, caffeic, ferulic, sinapic acids
Cauliflower	p-Coumaric, caffeic, ferulic, sinapic acids
Green cabbage	p-coumaric, caffeic, ferulic, sinapic acid
Chicory	Caffeic, ferulic, sinapic, p-hydroxy benzoic, vanillic acid
Garden cress	Vanillic, ferulic, sinapic acid
Horseradish	Vanillic, p-hydroxy benzoic, gentisic acids
Onion	Ferulic, p-hydroxy bezoic, protocatechuic, vanillic
Peas	p-hydroxy benzoic, ferulic, vanillic, syringic, p-coumaric
Potato	p-coumaric, caffeic, ferulic, sinapic, chlorogenic acid
Spinach	p-coumaric, ferulic acid

Phenolic acids have been known to possess sensory properties described as being astringent and bitter that are not considered very desirable in some products.

1.2.5.3. Glucosinolates

Glucosinolates (GSLs) constitute a well defined group of specialized plant metabolites. They are predominantly found in crucifers. Several other plant families in the same plant order as the Brasicaceae, the Capparales (e.g., the Capparidaceae, Moringaceae, Resedaceae, Stegnospermaceae, and Tovariaceae) have been found to possess glucosinolates. Table 5 represents glucosinolates identified in various food products.

A large body of epidemiological evidence indicates that the chemoprotective effects of Brassica vegetables against initiation of tumors caused by chemical carcinogens may be due to glucosinolates and their degradation products [54]. There is a considerable interest in recent years in optimizing GSL content and composition for plant protection and human health. GSLs are also responsible for the bitter acidic flavors of Brassicacea species. Their hydrolytic by-products such as isothiocyanates, nitriles, and thiocyanates are responsible for the hot and pungent taste.

Structurally glucosinolates are anions composed of thiohydroxymates carrying an S-linked β-glucopyranosyl residue and an O-linked sulfate residue, and with an amino acid derived, variable side chain (Figure 5). Based on the nature of side chain, they are broadly classified as alkyl, aromatic, benzoate, indole, multiple glycosylated and sulfur containing side chains. The enzyme myrosinase (β-thioglucosidase glucohydrolase; EC 3.2.3.1)-activated in damaged plant tissue and also present in the microflora of the human digestive tract converts these glucosinolates to a number of compounds including

thiocyanates, nitriles and isothiocyanates (Figure 5). These hydrolysis products, many of which possessing biological activity, vary depending on the plant species, side-chain substitution, cell pH, and cell iron concentration (55, 56].

Table 5. Glucosinolates in different food sources.

Name	Side chain (R)	Food source
Glucolepidin	Ethyl	Radish
Glucoputranjivin	Isopropyl	Radish
Sinigrin	2-Propenyl	Cabbage
Glucoiberin	3-Methylsulfinylpropyl	Cabbage
Glucocheirolin	3-Methylsulfonylpropyl	Cow\s milk
Epiprogoitrin	(2S)-2-Hydroxy-3-butenyl	Sea kale
Glucoraphanin	4-Methylsulfinylbutyl	Broccoli
Glucoibervirin	3-Methylthiopropyl	Cabbage
Glucoraphenin	4-Methylsulfinylbut-3-enyl	Radish
Glucoputranjivin	isopropyl	Drumstick
Glucocochlearin, Glucojiabutin	2-butyl	Drumstick
Isobutyl glucosinolate	2-Methylpropyl	Drumstick
4-(α-L-rhamnosyloxy)benzyl	benzyl	Drumstick
Glucosinalbin	4-Hydroxybenzyl	Drumstick
Glucoconringiin	2-Hydroxy-2-methylpropyl	Drumstick
Glucoalyssin	5-Methylsulfinylpentyl	Rocket
Glucotropaeolin	Benzyl	Drumstick, Cabbage
Glucocapparisflexuosain	Butyl	Cabbage
Gluconapoleiferin	2-Hydroxy-pent-4-enyl	Swede
Neoglucobrassicin	N-Methoxy-3-indolylmethyl	Cabbage
Glucobrassicin	3-Indolylmethyl	Cabbage
Glucocapparin	Methyl	Capers
Dehydroerucin	4-Methylthiobut-3-enyl	Daikon\s radish
Glucoerucin	4-Methylthiobutyl	Cabbage

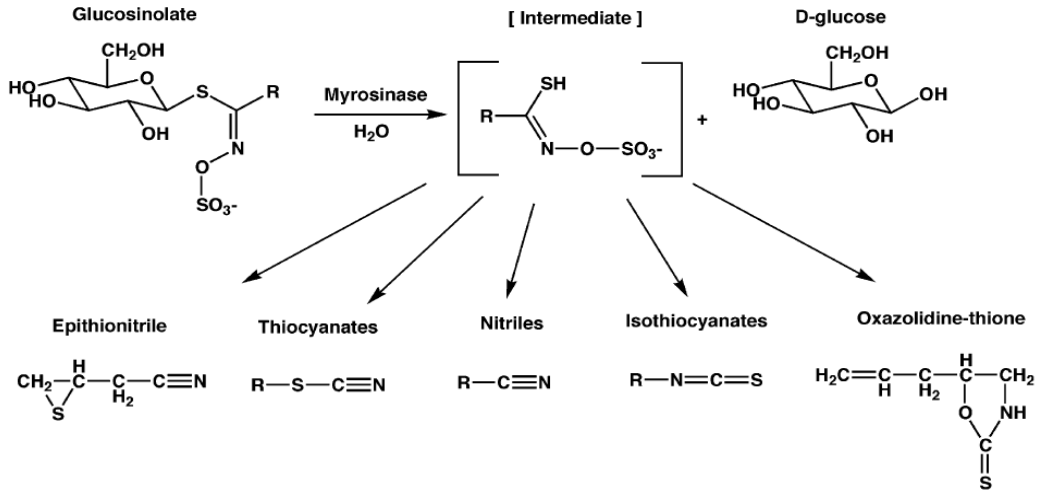


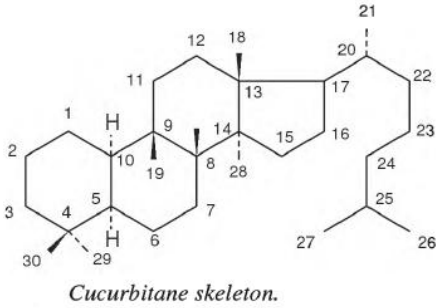
Figure 5. General structure of glucosinolates and their enzymatic degradation products. (Adapted from Rask et al., 2000 [57]).

In contrast to intact GLS, hydrolysis products are responsible for the characteristic aroma of Brassicaceae plants and have been of interest for their diverse biological activities, ranging from antimicrobial, antioxidant and anticancer activities [58]. Many of these have biocidal activity against a wide variety of organisms such as insects, plants, fungi, bacteria and human health effects [59]. For example, sulforaphane [1-isothiocyanato-4-(methylsulfinyl)butane], a degradation product of the glucosinolate glucoraphanin [4-(methylsulfinyl)butyl glucosinolate], is a potent inducer of phase II detoxication enzymes, that are strongly correlated with the prevention of certain types of cancer [60].

1.2.5.4. Triterpenoids

Triterpenes or triterpenoids are terpenes consisting of six isoprene units and having molecular formula $C_{30}H_{48}$. Triterpenoids are biosynthesized in plants by the cyclization of squalene, a triterpene hydrocarbon and precursor of all steroids [61]. They are used for

anti-inflammatory, analgesic, antipyretic, hepatoprotective, cardio tonic, sedative and tonic effects [61]. They also demonstrate antitumor efficacy in preclinical animal models of cancer [61]. They can further be sub classified into diverse groups including cucurbitanes, cycloartanes, dammaranes, euphanes, friedelanes, holostanes, hopanes, isomalabaricanes, lanostanes, limonoids, lupanes, oleananes, protostanes, sqalenes, tirucallanes, ursanes and miscellaneous compounds [61]. Among them, cucurbitacins are well-known for their cytotoxic behavior and broad range of bioactivities such as antitumor, anti inflammatory, antimicrobial, antihelminthic, and cardiovascular properties [62]. Cucurbitacins are oxygen containing compounds possessing double bonds and derived from cucurbitanes ($C_{30}H_{54}$) that have a triterpene hydrocarbon skeleton (Figure 6). They usually occur as β-2-monoglycosides, the sugar moiety being D-glucose or L-rhamnose. They possess the biogenetically unusual 10acucurbit-5-ene-[19(10-19b)-abeo-10a-lanostane] skeleton [63], which has mainly been reported in the Cucurbitaceae but is also known to occur in other plant families [63].



Cucurbitane skeleton.

Figure 6. Chemical structure of cucurbitane ($C_{30}H_{54}$).

1.3. Quality measurement of fresh-cut produce - Sensory and instrumental measurements

The quality of fresh-cut produce can be measured by sensory and instrumental methods. In general, sensory methods are more useful in developing new products and determining product standards while instrumental methods are superior in measuring quality on a routine basis [21].

1.3.1. Sensory methods of quality measurement

Sensory methods are very important for quality measurement of fresh-cut produce. Since human perception is involved in sensory testing, quality attributes are clearly defined in terms that are relevant to consumer acceptability. The sensory panels need to be extensively trained as highly variable results can be produced if training is inadequate. Trained sensory panels can be used to identify small differences in the samples processed with different treatments (Difference tests) or for descriptive analysis of a product. Descriptive analysis involves the development of a lexicon [64] which is the list of terms used as descriptors with their precise definitions. Lexicons can be extensive with up to 50 descriptors or with as few as 5 descriptors. Selection of a lexicon is followed by training of the panel to acclimatize them with the descriptors in order to ensure that the results are accurate and precise.

After completion of the training, the evaluation of the samples is conducted in partitioned booths using one of the several evaluation methods such as Spectrum [64] and Quantitative Descriptive Analysis (QDA; [65]). The primary differences between the two

techniques are that Spectrum uses standards for the descriptors and involves more extensive training than QDA. A more limited approach to descriptive analysis is the use of an experienced panel. Experienced panelists have had some training on similar descriptors in the past, but the panel is usually provided with a limited number of descriptors for evaluation. The panel is convened with a few training sessions primarily to ensure that the panelists are familiar with the terminology. Usually two to five judges evaluate the samples independently of each other to prevent bias.

However, to determine the preference (which samples are preferred over others) of a food product, large numbers of naïve panelists are generally used [66]. The preference tests give an idea about what consumers like and what they do not like.

The most frequently used evaluation scale is the 9-point hedonic scale [64]. Some more useful scales include the 5-point willingness to purchase [67] and the 3-point acceptability scale [68]. Table 6 represents the sensory scales generally employed in the food industry for deciding the acceptability of a product.

However, the use of a single sensory technique provides limited information. Integration of two or more techniques can be a powerful tool in the quality evaluation of fresh-cut produce [69].

To summarize the main points, sensory analysis helps to determine which attributes are important to the consumer. Difference tests can determine if individual units are noticeably different, and sensory descriptive analysis can identify the attributes that cause the differences. When carefully coordinated, the sensory tests can be very effective in developing new products and establishing quality standards.

Table 6. Sensory scales used in the evaluation of food quality

Hedonic	Purchase	Acceptabilty
1-Dislike extremely	1-Definitely would not	1-Unacceptable
2-Dislike very much	2-Probably would not	2-Acceptable
3-Dislike moderately	3-Might or might not	3-Tastes great
4-Dislike slightly	4-Probably would	
5-Neither like nor dislike	5-Definitely would	
6-Like slightly		
7- Like moderately		
8- Like very much		
9- Like extremely		

1.3.2. Instrumental methods of quality measurement

Apart from sensory analysis which is a crucial step in product development, a wide range of instrumental techniques for quality assessment of fresh-cut produce in terms of color, appearance, flavor, texture, and nutritional quality are available. These techniques, also termed as objective methods, are advantageous in a way that they tend to provide accurate and precise results. The results of instrumental tests can generally be related directly to chemical and physical properties allowing the investigator to gain a mechanistic understanding of observed differences. Instruments tend to be more sensitive to small differences between samples and may be able to detect trends in quality loss before they can be detected by humans [70]. Large amounts of data can be generated by using instruments without any fatigue or loss of sensitivity/ alertness towards analysis. This

attribute makes them excellent monitors in Quality Control operations. Instrumental methods of measuring color, texture, aroma, and flavor in fruits and vegetables as described by Kader [71] are listed in Table 7.

Table 7. Instrumental methods for determination of vegetables quality

S.No.	*Quality Attribute*	*Objective method of measurement*
1	Color	Color charts, reflectance and transmittance colorimeters, pigment extraction and spectrophotometers
2	Texture	Texture analyzers-compression, shearing, analysis of solids, moisture
3	Aroma	Gas chromatograph, enzymes
4	Flavor	Refractometer, pH meter, determination of acidity and sugars, enzymes, amines, bitter alkaloids or glucosides
5	Nutritional value-Vitamin A, B, C, E, polyphenolics, carotenoids, glucosinolates	HPLC and spectrophotometric methods

1.3.2.1. Color

Color may be determined using nondestructive methods based on visual or physical measurements. These methods are based on evaluation of either the light reflected from the surface of a product or transmitted through it. There are three components necessary for the perception of color - 1) a source of light, 2) an object that modifies light by reflection or transmission and 3) the eye/brain combination of an observer. Easy and fast

methods such as use of simple color charts and dictionaries are routinely used in the field, packing house, fresh-cut processor facility or retail store. They have the advantage of requiring no specialized equipment, but may be standardized through the use of color charts or discs. However, human differences in perception might give erroneous results. Inadequate or poor quality of the available light may also affect accuracy [18]. On the other hand, instrumental methods are less variable and can be used to measure small differences; however, they may be slower than the sensory measurements.

Each pigment in vegetables corresponds to a primary hue—red, blue, and green [72].

The Commission Internationale de l'Eclairage (CIE) or International Commission on Illumination is the international body that governs the measurement of color. Color space may be divided into a three-dimensional (L, a and b) rectangular area (Figure 7) such that L (lightness) axis that goes vertically from 0 (perfect black) to 100 (perfect white) in reflectance or perfect clear in transmission [73]. The "a" axis (red to green) considers the positive values as red and negative values as green; 0 being neutral. The "b" axis (blue to yellow) expresses positive values as yellow and negative values as blue; 0 being neutral.

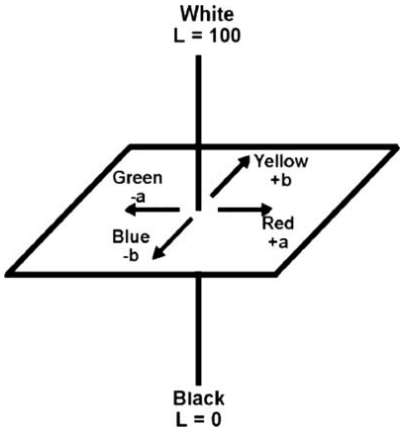


Figure 7. Diagram depicting three dimensional L, a and b color space

Color may be determined instrumentally using either colorimeters or spectrophotometers. Colorimeters give measurements that can be correlated with human eye-brain perception, and give tristimulus (L, a and b) values directly [73]. Colorimeters are typically quite rugged and desirable for routine quality control measurements. Spectrophotometers provide wavelength-by-wavelength spectral analysis of the reflecting and/or transmitting properties of objects, and are more commonly used in research and development laboratories [73].

Pigments of vegetables may also be analyzed quantitatively by extraction with specific solvents, filtration, and the use of various methods based on spectrophotometry. Separation using reversed phase high performance liquid chromatography (HPLC) may be useful prior to measurement of absorption of light in the UV/visible wavelength spectrum. Colorimetric methods are based on the Lambert-Beer law which describes the relationship between the concentration of a substance and its color intensity:

$E = \varepsilon' lc$

The extinction coefficient E is proportional to ε', the molar extinction coefficient of the substance, l, the length of the light path in centimeters, and c, the concentration in g l^{-1}.

1.3.2.2. Texture

Most methods used for the evaluation of the textural properties of vegetables comprise a wide range of simple and rapid tests, including puncture, compression, extrusion, shear, and others. The puncture test, which is a force measuring method that has the dimensions mass, length, and time, is the most frequently used method for textural evaluation. It

consists of measuring the force and/or deformation required to push a probe or a punch into a food material to a depth that causes irreversible damage or failure. Puncture probes of a specific diameter may be easily fitted to laboratory-scale instruments such as the Maturometer, the Instron, and the Texture Technologies TAXT2 machine for more controlled measurements [74].

1.3.2.3. Flavor- Aroma and Taste

Flavor may be evaluated with either instrumental or sensory methods, however, sensory methods are the most critical to this particular attribute. Therefore, flavor may be the most challenging quality attribute to both measure and correlate to consumer acceptability.

1.3.2.3.1. Analysis of aroma compounds

There are several challenges in the analysis of aroma compounds. It involves their isolation from a given product, further separation, quantification and then correlating their odor to the chemical structure. Odor molecules can be found in any class of chemical compounds and thus can have widely varying structure and polarity. Further, these compounds are labile and susceptible to chemical changes. For example, terpenes can undergo photooxidation/ rearrangements in the presence of light or unsaturated compounds can undergo polymerization in the presence of air. Such changes are of concern during sample preparation as they generate artifacts. The problem is further compounded by their occurrence in minute quantities (ppb-ppt) requiring sensitive and sophisticated instruments for their detection and analysis. Contribution of an odorant to the total aroma of a food product is additionally dependent on its threshold, which results

in even a compound present in minute amount having a greater contribution to the odor than constituents present in very large concentration. Two terms namely "odor impact compounds" and "contributory odor compounds" have thus been used to define top odor notes. The former refers to those compounds that actually contribute to the typical odor of a product while the latter modify the typical odor to a pleasant and acceptable note.

1.3.2.3.2. Isolation of aroma principles

Aroma isolation from a given matrix involves crushing, homogenizing, blending or extracting the matrix with minimum loss in these constituents. Fresh materials contain active enzymes in their tissues that can alter the aroma profile once cellular disruption has occurred. The complex nature of the food matrix containing proteins, fats or carbohydrates makes the process of aroma isolation complicated. Solvent extraction using organic solvents at room or sub ambient temperatures is one of the most common and conventional method for extraction of aroma compounds. The nature of the solvent used, polar or non-polar, depends on the type of compounds to be isolated and identified. Drawbacks of this method, however, are the co-extraction of non-volatile constituents posing problems in recovery of volatile odors and the solvent impurities interfering in the analysis, and falsely identified as a sample constituent, particularly when large amounts of solvent are used. Supercritical fluids are now being used particularly in supercritical fluid extraction, thus avoiding the problem posed by the use of organic solvents. SFE uses variety of fluids (typically, CO_2 possibly modified with very low amounts of organic solvents as modifiers), high pressures (2000-4000 psi) and elevated temperatures (50-

150°C). The method produces clean extracts with no additional concentration for gas chromatographic (GC) analysis.

Steam distillation techniques such as simultaneous distillation extraction (SDE) are widely used for the isolation of aroma from vegetables. However, isolation of odor molecules using SDE can bring about drastic changes in odor quality such as formation of cooked odors. High vacuum low temperature distillation, solid phase micro-extraction, microwave-assisted extraction, pressurized fluid extraction and head space techniques are generally employed for extracting the fresh aroma of the vegetables to avoid artifact formation due to thermal treatment in steam distillation techniques.

High vacuum distillation is a mild technique operated at or below the ambient temperature wherein the potential loss of very low boiling volatiles is minimized. Artifact formation observed in many other distillation techniques is eliminated. The method provides for true odor of a given product. However, only low yield of aroma concentrates are obtained compared to steam distillation technique and thus is generally used for the isolation of labile compounds or when the aroma impact components have to be identified or confirmed.

Microwave-assisted extraction involves extraction using microwave transparent solvents. Contents of the cells broken by internal heating at microscopic level by microwave energy come into contact with surrounding cool solvents avoiding thermal degradation of labile odors. However, concentration of the isolate prior to GC analysis is required.

Headspace analysis is another very useful technique for rapid identification of volatile aroma in headspace above a food sample. Two methods generally employed are the static

and dynamic headspace techniques. Static headspace involves enrichment of volatiles in a closed chamber and is generally used when low volatile emitting samples are to be investigated. The method has the disadvantage of long sampling times. In order to reduce sampling time and to obtain better yields in static headspace, solid phase microextraction (SPME) is currently employed for collection and further identification by GC. The method is fast, effective, simple and is based on adsorption-desorption technique using an inert fiber coated with different types of adsorbents varying in polarity and thickness and used depending on the application demanded. Non-polar volatile compounds are effectively extracted with non-polar fiber coatings such as polydimethylsiloxane (PDMS) while polar volatiles can be extracted with PDMS/divinylbenzene or PDMS/Carboxene polar fibers. The fibers equilibrated with headspace volatiles are directly transferred to the GC injection port where the compounds are desorbed thermally. This eliminates solvent-mediated desorption, thereby reducing the risk of solvent contamination. Some of the limitations of SPME include (i) no repeated injections are possible, and (ii) the low amount of material adsorbed on SPME fibers makes the method amenable only to GC analysis but not for structure elucidation of unknown compounds. Consistent sampling time, temperature and sample volume are crucial to obtain comparable results.

In dynamic headspace, a continuous air stream is passed over the headspace, and the odorous compounds carried along with air are trapped on to porous polymeric traps such as Tenax (2,6- diphenyl-p-phenylene oxide) and Porapack (ethylvinylbenzene-divinylbenzene) or carbon-based adsorbents such as activated charcoal, carbon molecular sieves and graphitized carbon black. The volatiles can be directly desorbed from the

adsorbent into the GC in case of porous polymers while in case of carbon-based adsorbents the volatiles are generally eluted with small volumes (30-40 µl) of organic solvents. Although, Tenax has a lower capacity than Porapack, it has higher thermal stability and thus is particularly suited for thermal desorption of volatile compounds in GC analysis. A combination of tenax and charcoal with high adsorbing capacity have been employed for trapping full range of odorants varying widely in their polarities. Dynamic headspace permits collection of large amount of volatile aiding in identification of unknown structures.

1.3.2.3.3. Separation and detection of aroma compounds

As discussed earlier, a volatile odor extract is generally made up of an aroma bouquet containing anywhere from a few to several different constituents present in varying amounts. The individual components have to be separated from the mixture to facilitate their identification. The most commonly used method of separation is the chromatographic technique based on adsorption / partition of constituents between two phases. Among the chromatographic techniques, gas liquid chromatography (GLC) is the most efficient technique for the separation, identification and quantification of volatile organic compounds. The separations are affected by both sample preparation and isolation procedure. The nature of the stationary phase in the column has a distinct impact on separation efficiency. Commonly used stationary phases are the non polar dimethyl polysiloxanes (DB-l, DB-5, CPSil 5, SE-30 and OV-1) and the more polar polyethylene glycol polymers (Carbowax^{TM} 20 *M,* DB-Wax and HP 20M). For different stationary

phases, retention index data such as kovats index system have been developed to facilitate compound characterization and identification. Detection of peaks can be carried out using two types of detector. First type include the flame ionization detector (FID) and the thermal conductivity detector (TCD) that provide the retention times while the second type include the mass spectrometer (MS) and the Fourier transform infrared (FT-IR) spectrometers that aid in obtaining structural information. FID is a highly sensitive detector (0.05 – 0.5 ng per compound) and is based on detection of ions formed when organic compounds are burnt in a flame, while TCD, a less sensitive detector, operates by differential thermal conductivity of gaseous mixture. MS with a sensitivity of 0.1 - 1 ng per compound, relies on generation of positively charged molecules/and molecule fragments from compounds separated on the GC column. Several comprehensive mass spectral libraries (WILEY, NIST MS data base) have been established and are currently used in EI-MS searches for tentative compound identification. Unambiguous identification can, however, be achieved by comparison of mass spectrum with that of authentic standards analyzed on the same column and determination of kovats indices on at least two columns with different polarities. Multi dimensional GC, **w**herein constituents of volatile isolates are separated by sequentially passing through two successive columns, is used for separation of complex isolates such as essential oils.

Only a small fraction of the large number of volatiles in a given aroma isolate actually contributes to the odor. The distinction between odor active compounds and the whole range of volatiles present in an isolate from a food is thus an important task in odor analysis. Sniffing the gas chromatographic effluents of an isolate in order to associate

odor activity with eluting compounds is an interesting approach. Principal analytical technique used in this regard is the gas chromatography-olfactometry (GC-O) technique that aids in assessing the aroma potential of a compound. GC-MS-O provides a means of identification of compounds. First formal approach to determine contribution of a volatile compound to the overall odor is based on its odor activity values (OAV) which is defined as the ratio of concentration of the volatile compound to its odor threshold. The so-called odor impact compounds can be distinguished from other volatiles by their high OAV. Among the GC-O techniques, the dilution sniffing methods namely Aroma Extraction Dilution Analysis (AEDA) and the Combined Hedonic Aroma Response measurements (CHARM) are routinely used for identification of odor active constituents. Both methods involve sequential dilution of samples. In AEDA, the most widely used method, each dilution is sniffed after injection on to the GC until no significant odor is detected. The result is then expressed as a flavor dilution (FD) factor which is the ratio of concentration of the odorant in the initial extract to its concentration in the most dilute extract in which an odor was detected. However, the method requires evaluation by a trained panel. The methods thus lack reproducibility due to variability within and between sensory panelists. A more recent method based on frequency of detection of a given odor, rather than perceived intensity termed as Olfactory Global Analysis, eliminates the drawback of dilution techniques. The repeatability of the method has been demonstrated to be satisfactory despite the use of untrained sensory panel. The method is also twice as fast as other GC-O techniques. A group of assessors is, however, a prerequisite for a reliable

GC-O analysis and thus the results can be systematically affected due to decreasing alertness of the assessor.

1.3.2.3.4. Isolation and identification of bound aroma glycosides

The glycosidic aroma precursors are isolated from plant extracts (aqeuous or aqueous methanol) by selective retention on C-18 reversed phase adsorbent [75] or amberlite XAD-2 resin [76], followed by the desorption of the retained glycosides using EtOAc or MeOH. The eluate so obtained is generally subjected to acidic or enzymatic hydrolysis and the liberated aglycones are analyzed by GC/MS for identification. However, to characterize intact glyco-conjugates, sophisticated steps of chromatographic separations need to be followed. For preliminary separations, LC techniques are generally suited, such as preparative HPLC and size exclusion chromatography. The isolates are then subjected to repeated purification steps before identification. Purified compounds are identified based on spectroscopic techniques such as NMR, IR, MS, or chiroptical methods or analysis of hydrolysis products by GC/MS.

1.3.3. Analysis of bioactive phytochemicals

The phytochemicals may be extracted depending on their water or lipid solubility and are typically analyzed using HPLC. Major anti-oxidant phytochemicals such as polyphenolic compounds like phenolic acids, flavonols, flavones or the flavonoids, are extracted in methanolic or ethanolic extract of the tissue, which after preliminary cleanup are subjected to HPLC or LC/MS analysis for separation and identification of the compounds [77]. In the case of colored phytochemicals like anthocyanins, it is possible to estimate its content by measuring the intensity of color or a/b value with a colorimeter, but such a

physical method is not available for most nutrients. Glucosinolates generally require hot aqueous alcohols such as methanol: water (70:30) for their isolation from plant materials in order to prevent their hydrolysis by myrosinase [88]. A prior separation into groups normally precedes their identification and quantification by HPLC-MSn. Presence of sulfate groups facilitates binding of these compounds to an anion exchange column and thus allows separation of either the intact GSLs or "desulfo" derivatives after enzymatic desulfation [78]. Direct analysis of volatile isothiocyanates and nitriles produced from GSLs by GC/MS can also provide proof of the presence of corresponding GSL in intact plant. On the other hand, lipophilic bioactive components such as carotenoids are generally extracted using non-polar solvents such as petroleum ether, hexane, THF, methanol, ethanol, or combinations of various solvents in different proportions. The identification is usually carried out by Diode Array Detector (DAD) or MS. Most of the vitamins are analyzed using standard AOAC methods.

1.4. Different methods for preservation of fresh-cut produce

A wide range of preservation techniques are available in food industry. Disinfectants presently employed in commercial processing lines, such as chlorine has limited effect (approx. 1 log reduction) on microbial populations and cannot be relied to eliminate pathogenic microorganisms like *L. monocytogenes*. These disinfectants cannot prevent damage due to elevated respiration and transpiration rates of minimally processed products. In addition it is believed that they may form carcinogenic derivatives in water (e.g. chloramines and trihalomethanes) [79]. Therefore, there is a need for research on

alternative treatments suitable for use on fresh-cut products. New protocols for maintaining quality while inhibiting undesired microbial growth for both the production facilities and distribution chains, are thus needed.

Newer methods available for preservation of RTC products can be classified in two categories- chemical and physical. Chemical methods involve use of chlorine dioxide, organic acids, hydrogen peroxide, calcium based solutions, ozone, electrolyzed water and natural preservatives. Under physical methods of food preservation, high pressure processing, pulsed white light, ultra violet light, pulsed electric field, oscillating magnetic fields and ionizing radiation are the recent techniques which are being used widely.

1.4.1. Chemical preservatives

Among the chemical methods, chlorine dioxide with a better oxidation capacity than chlorine, hydrogen peroxide, a strong oxidizing agent and ozonated water has been used for reducing microbial populations and shelf life extension of fresh produce. Efficacy of ClO_2 in the inactivation of *Listeria monocytogenes* and *Salmonella typhimurium* [80], and H_2O_2 solution in reducing microbial populations on fresh-cut bell peppers, cucumber, zucchini, cantaloupe, and honeydew melon, without alteration in sensory characteristics [81] have been reported. Although, antimicrobial activity of ozone is widely known, there is little information available about its efficacy against food borne pathogens. Higher corrosiveness of ozone, its negative effects on easily oxidizable compounds such as vitamin C and carotenoids; and initial capital cost for its generation are the main disadvantages in its use compared to other chemical preservatives.

Calcium is extensively used to extend the shelf life of fruits and vegetables. It helps maintain the vegetable cell wall integrity by interacting with pectin to form calcium pectate. Different salts of calcium used in food industry include calcium chloride, calcium lactate and calcium propionate. Antibacterial properties have been reported for calcium propionate during the treatment of honeydew melon, due to its ability to uncouple microbial transport processes [82].

Acidic electrolyzed water (pH 2.1-4.5) has a strong bactericidal effect against pathogens and spoilage microorganisms. It is more effective than chlorine due to its high oxidation-reduction potential (ORP). A higher effectiveness of electrolyzed water in reducing viable aerobes than ozone on whole lettuce has been demonstrated [83]. No adverse effects were noted on the w.r.t. surface color, pH or general appearance of fresh-cut vegetables.

The reluctance of consumers towards the use of chemical preservatives in recent years has resulted in the use of natural antimicrobials as preservatives. Organic acids such as lactic, citric, acetic and tartaric acids are used as strong antimicrobial agents against psychrophilic and mesophilic microorganisms in fresh-cut fruits and vegetables. The antimicrobial action of organic acids is due to pH reduction in the environment, disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid.

1.4.2. Physical methods of preservation

Several physical methods are currently employed for preservation of minimally processed plant produce. Modified atmosphere packaging (MAP) for example aims at producing low O_2 and high CO_2 in the atmosphere surrounding fresh cut produce. These conditions

reduce respiration rate thus delaying senescence and extending shelf life. In passive MAP the package is sealed under normal atmospheric conditions and desired composition is obtained due to produce respiration and film gas permeability. In active MAP the package is flushed with a gas mixture of preset composition and once closed, no further control of the gas composition is exercised. However, expensive equipment and materials (gases and packaging) are required for MAP generation.

Ultraviolet (UV) light is another physical treatment widely employed in industry. UV irradiation causes up to 4 log cycle reduction in bacterial, yeast and viral counts by inducing DNA damage. Major advantage of this technique is the availability of relatively inexpensive and easy to use equipment. Among the other technologies, treating products with millisecond pulses (1–20 flashes/sec) of broad spectrum white light, about 20,000 times more intense than sunlight holds promise. Pulsed white light inactivates microorganisms by combination of photochemical and photothermal effects, requires very short treatment times and has a high throughput. The above methods, however, have lower efficiencies due to their lower penetration and are thus mostly used for surface sterilization.

1.4.2.1. Radiation Processing

Radiation processing is a promising technology for improving the shelf life of fresh-cut produce. Treatment of food products by ionizing radiation is a physical process involving direct exposure to electromagnetic γ-rays, X-rays or electron beam for improvement in food safety and shelf life. It is a non thermal technology that effectively

eliminates food-borne pathogens in various foods including fresh vegetables without compromising the nutritional properties or sensory qualities of food [84].

Gamma irradiation can be employed for inhibition of sprouting, delay in ripening, killing of insect pests, parasites, pathogenic and spoilage microorganisms (Table 8). Radiation by its direct effect on macromolecules and indirect effect through radiolysis of water bring about alteration in the structure of essential bio-molecules present in insects, parasites, and microorganisms, thus reducing their chances of survival.

In 1980, Joint Expert Committee of Food and Agriculture Organization / International Atomic Energy Agency / World Health Organization on Food Irradiation concluded "The irradiation treatment of any food commodity up to an overall average dose of 10 kGy presents no radiological, microbiological or toxicological hazard." As a result, toxicological testing of foods so treated is no longer required. Food irradiation is now legally accepted in many countries. In 1997, one more expert group constituted by WHO, reaffirmed the safety of food irradiated at doses above 10 kGy. Consequently, in 2003 Codex Alimentarius Commission revised its Codex General Standard for Irradiated Foods to set standards for irradiation of foods worldwide. Agreements on Sanitary and Phytosanitary (SPS) Practices and Technical Barriers to Trade (TBT) under the World Trade Organization (WTO) have provided for adoption of irradiation as an SPS measure in international trade under the principle of equivalence. Thus, irradiation can be applied to overcome quarantine barriers, and to hygienize products for international trade.

The range of dose commonly employed in various food irradiation applications to achieve different objectives can be classified into three groups: low dose (< 1 kGy), medium dose

(1-10 kGy) and high dose (> 10 kGy) applications. Table 9 lists the food items approved for food irradiation under FSSAI Indian rules.

Table 8. Various applications of food irradiation (Table adapted from FSSAI (www.fssai.gov.in))

S. No.	Food applications of various radiation doses	Dose (kGy)
1.	**Low Dose Applications**	
	• Sprout inhibition in bulbs and tubers	0.03 – 0.15
	• Delay in fruit ripening	0.25 – 075
	• Insect disinfestations and elimination of food borne pathogens	0.07 – 1.00
2.	**Medium Dose Applications**	
	• Reduction of spoilage microbes to improve shelf-life of meat, poultry and sea foods under refrigeration	1.50 – 3.00 3.00 – 7.00
	• Elimination of pathogenic microbes in fresh and frozen animal foods	7.00 – 10.00
	• Reducing number of micro-organisms in spices to improve hygienic quality	
3.	**High Dose applications**	
	• Sterilization of packaged meat, poultry and their products which are shelf-stable without refrigeration	25.00 – 70.00 25.00 – 70.00
	• Sterilization of hospital diets	25.00 – 70.00
	• Product improvement as increased juice yield or improved rehydration	

Table 9. Food items approved for radiation processing in India under FSSAI rules

Name of Food	Purpose	Dose (kGy)	
		Min.	Max.
Onion	Sprout inhibition	0.03	0.09
Potato		0.06	0.15
Ginger, garlic		0.03	0.15
Shallots (small onion)		0.03	0.15
Mango	Disinfestation (Quarantine)	0.25	0.75
Rice, semolina (rawa), whole wheat flour (atta), and maida	Insect disinfestation	0.25	1.00
Raisins, figs and dried dates		0.25	0.75
Pulses		0.25	1.00
Dried seafoods		0.25	1.00
Meat and meat products including chicken	Shelf-life extension and pathogen control	2.50	4.00
Fresh seafood	Shelf-life extension under refrigeration	1.00	3.00
Frozen seafood	Pathogen control	4.00	6.00
Spices	Microbial decontamination	6.00	14.00

1.4.2.2. Advantages of Radiation Processing

• It is an effective alternative to chemical fumigants that endanger human health and environment.

• At recommended doses it maintains fresh-like character, sensory qualities, texture, nutritive value and appearance of food.

• Does not produce any toxic residues in food.

• Unlike chemical fumigants irradiation can be carried out in pre-packaged foods and hence no risk of post-irradiation contamination.

• Being highly penetrating and effective, large volumes of foodstuffs can be treated very efficiently.

• Radiation processing is an eco-friendly treatment and does not pollute environment.

1.4.2.3. Effect of gamma irradiation on shelf-life of RTC vegetables

Shelf life of a fresh produce is determined by its microbial status as well as by its sensory attributes such as color, texture and flavor (aroma and taste). Gamma irradiation has been reported to cause minimal modification in these quality attributes of fresh-cut produce. However, the levels of modification in the quality attributes might vary depending on the raw material used and radiation dose delivered [85]. Studies conducted on irradiated RTC vegetables have demonstrated no significant changes in the appearance, color, texture, taste and overall acceptability after seven days of irradiation (1 kGy) as compared to the control, when stored at low temperatures (4 - 10 °C) [86]. In contrast, adverse effects of radiation treatment were observed on color, taste, flavor and texture by the sensory panel, in pre-cut irradiated tomatoes (*lycopersicon syn. L. esculentum*), cantaloupe melon (*Cucumis melo*), and watermelon (*Citrulus lanatus*) [87]. 88, Bibi et al. [88] also reported

a decreased firmness and lower appearance scores for irradiated tomatoes (*L. esculentum*) as compared to controls during storage.

An improvement in the quality of fresh-cut produce by radiation processing has also been reported by several researchers. Radiation treated products such as conventional chicory had a better general acceptability when irradiated at doses 1.2 and 2 kGy [89]. The overall acceptability of carrots was found to be higher up to a radiation dose of 2 kGy as compared to corresponding controls [90]. The better quality retention in general, has been attributed to the decreased physiological and microbial decay in the radiation processed samples. Slight increase in sweetness was observed in radiation-processed carrot (*Daucus carota*) at a dose of 2 kGy. Thus, radiation processing may be employed for improving the shelf life of fresh-cut produce with minimum or no alteration in the sensory quality.

1.4.2.4. Microbial status of irradiated RTC vegetables

One of the main uses of radiation processing is to control the microbial spoilage of food products. Ionizing radiation causes radiolysis of water producing reactive hydroxyl radical. Hydroxyl radicals are extremely reactive and attack and damage cellular components, especially DNA. Damage to genetic material results in inhibition of microbial growth. Significant reduction in the microbial load consequent to radiation processing has been widely reported in literature. Irradiation of broccoli and mung bean sprouts at 1.0 kGy resulted in reduction of approximately 4.88 and 4.57 log CFU/g, respectively, of a five-strain cocktail of *L. monocytogenes*. The effects of low-dose irradiation on the microbiota of pre-cut tomato (lycopersicon syn. L. esculentum) were investigated by Mohacsi-Farkas et al. [87]. Doses of 1–3 kGy were able to reduce

considerably the microbiological contamination in tomato. The low dose irradiation reduced the viable cell count of *Listeria monocytogenes* by 2 log-cycles. A dose of 1 kGy reduced the viable cell number by more than 5 log-cycles. These results indicate that radiation processing has been successfully employed for microbial decontamination of the fresh-cut fruits and vegetables. The technology therefore could result in improved shelf-life of the fresh-cut produce.

1.4.2.5. Effect of radiation processing on phytochemicals

Levels of phytochemicals in plants vary depending on various conditions. Exposure to radiation sources, wounding, storage at low temperatures, and/or exposure to extreme temperatures [91] can lead to changes in the nature/content of bioactive phytochemicals. In terms of radiation exposure, the changes depend on the applied dose (usually low and medium doses have insignificant effects on phytochemicals), the sensitivity of the phytochemicals towards irradiation, and the effect of irradiation on precursors that might be responsible for the production and/or the accumulation of phytochemicals in the plant. Radiation processing has been shown to either increase or decrease the antioxidant content of plant produce, depending on the dose delivered, time of exposure and the nature of raw material to be irradiated.

The enhanced antioxidant capacity of a plant after irradiation is mainly attributed either to increased enzyme activity (e.g., phenylalanine ammonia-lyase and peroxidase activity) or to the increased extractability from the tissues [85, 91]. It has been also proposed that gamma radiation is capable of breaking the chemical bonds of polyphenols, resulting in

the release of low molecular weight water soluble phenols, leading to an increase of antioxidant rich phenolics [92].

A positive correlation between phenolics and antioxidants has been observed in several cases. The increased antioxidant capacity has been correlated with tissue browning resulting from an action of polyphenol oxidase (PPO) [93]. Fan et al [94] while working on lettuce, reported that the free radicals generated during irradiation might act as stress signals and may trigger stress responses in lettuce, resulting in an increased antioxidant synthesis. Fan et al. [95] studied the effect of radiation treatment (0, 0.5, 1 and 2 kGy) and storage (up to 8 d) at 7-8 °C on phenolics content, antioxidant capacity and tissue browning of romaine lettuce, iceberg lettuce and endive. Their results revealed an enhancement in the phenolic content and antioxidant capacity of all vegetables at 4 and 8 d after irradiation. The increase in the total antioxidant capacity was attributed to increased phenolic synthesis.

Reduction in contents of antioxidants has also been reported by various researchers. In general, the decrease in antioxidants has been attributed to radiation-induced degradation or the formation of free radicals [96]. Breitfellner et al [97] have reported that gamma-irradiation (1 to10 kGy) of strawberries lead to the degradation of phenolic acids like cinnamic, p-coumaric, gallic, and hydroxybenzoic acids.

In recent years there has been an increased demand for RTC vegetables among Indian consumers. However, very less work has been carried out on the possibility of using gamma radiation processing to extend the shelf-life of minimally processed Indian vegetables. It was therefore of interest to determine the feasibility of using radiation

processing for extending the shelf life of some commonly used RTC Indian vegetables and understand changes in major bioactive principles in these products.

1.5. Vegetables selected for the present work

Three popular Indian vegetables namely ash gourd (*Benincasa hispida*), pumpkin (*Cucurbita pepo*) and drumstick (*Moringa oleifera*) were selected for the present study. Besides their nutritive value, these vegetables have also been used traditionally for their pharmacological and medicinal value.

1.5.1. Ash gourd (*Benincasa hispida*)

Ash gourd (*Benincasa hispida*), a member of the family Cucurbitaceae and native of Asia, is one of the most important vegetables grown primarily for its fruits and usually recognized for its nutritional and medicinal properties. It is also known as winter melon, ash gourd, ash pumpkin, white gourd, white pumpkin, gourdmelon, tallow gourd, wax gourd and Chinese watermelon [98]. The average production of the fruit of this green vegetable is around 18.5 t/ha [99]. India is the second largest producer of this vegetable after China [100]. The plant is monocious vine type bearing large green fruits that are either allowed to spread on the ground or trained to climb a support. It takes from 6 to 9 weeks for the yellow flowers to develop after seed germination and the fruit matures after a period of 2-3 months.

The fruit has been claimed to be a high quality vegetable, based on the Index of Nutritional Quality (INQ) data [101]. It is a good source for natural sugars, amino acids, organic acids, minerals and vitamins. Table 10 depicts the proximate composition of

immature and mature fruit. Moisture contents are quite high in the fruit accounting for 96 % of the edible portion of the mature fruit. Table 11 summarizes various vitamins and minerals content reported in mature ash gourd fruit. It contains an appreciable amount of vitamin C ranging from 1.35 – 68.00 mg/100 g while the content of thiamin is lowest (0.02 – 0.04 mg/100 g). Potassium (K) and calcium (Ca) are the major minerals present in ash gourd with concentrations ranging from 77 – 131 mg/100 g and 5 – 23 mg/100 g respectively. Both these minerals play a crucial role in maintaining the electrolytic balance of the body fluid.

Table 10. Proximate composition of immature and mature ash gourd fruit (g/100g of edible portion) (Zaini et al., 2010 [100])

	Immature fruit	Mature fruit
Moisture	93.80 – 95.80	94.50 – 96.80
Protein	0.47 – 0.70	0.30 – 0.50
Carbohydrate	2.69 – 2.70	1.10 – 4.00
Fiber	0.56 – 201.	0.50 – 1.50
Fat	0.00 – 0.02	0.00 – 0.30
Ash	0.45 – 0.70	0.27 – 0.45

The vegetable finds use as a medicine in the traditional Asian system of medicine system such as the Ayurveda. It is an important ingredient of “Kusmanda lehyam” (Ayurvedic medicine) [102]. It a rich source of various functionally important bioactives such as triterpenes, phenolics, sterols, and glycosides. It has been widely used for the treatment of epilepsy and other nervous disorders [103]. The juice and extract of the fruit is known to possess significant anti-ulcer, anti-depressant and diuretic activities, and provide

protection against histamine-induced bronchospasm [101]. Common medicinal and pharmacological properties of the vegetable are listed in table 12.

Table 11. Vitamins and minerals profile of mature ash gourd fruit (100 Zaini et al., 2010)

		Content (mg/100 g of edible portion)
Vitamins	Vitamin C	1.35 – 68.00
	Thiamin	0.02 – 0.04
	Riboflavin	0.02 – 0.31
	Niacin	0.20 – 0.46
Minerals	Sodium (Na)	0.14 – 6.00
	Potassium (K)	77.00- 131.00
	Calcium (Ca)	5.00 – 23.32
	Iron (Fe)	0.20 – 0.49

Owing to its popularity, many studies on ash gourd have been reported. Some volatile compounds have been identified in the ash gourd with aliphatic alcohols and carbonyl compounds as the major class [104]. Volatile compounds of the beverages prepared from this vegetable were analyzed by Sikorski [105] and Wu et al. [106]. Pyrazines were found to be major compounds detected in beverages which were presumably formed by the Maillard reaction during extraction of juices.

Table 12. Some common medicinal and pharmacological properties of different parts of ash gourd (100 adapted from Zaini et al., 2010 [100])

Vegetable part	Medicinal and pharmacological properties
Pulp	Anti-inflammatory, anti-ulcer, anti-depressant, anti-histaminic, antioxidant, anti-compulsive, anti-diarrheal and anti-obesity activities; beneficial effects in allergic inflammation, insanity and epilepsy; preventive and curative effects in nervous disorder, intestinal worms, jaundice, diabetic, leucorrhoea, stomach and bile problems; potential uses as diuretic, laxative, aphrodisiac, clearing heat and detoxificant; used for Alzheimer disease treatment, facial eruption, inhibition of angiotensin converting enzyme (ACE).
Seed	Anti-angiogenic, anti-tumor, antioxidant, anti-nociceptive, and anti-pyretic activities; soporific potential, and beneficial effects for brain and liver; used for the treatment of syphilis, cardiovascular diseases, inhibition of angiotensin converting enzyme (ACE), expel intestinal worm and softening or soothing the skin.
Peel	Anti-angiogenic, anti-tumor, antioxidant, anti-nociceptive, and anti-pyretic activities; soporific potential, and beneficial effects for brain and liver; used for the treatment of syphilis, cardiovascular diseases, inhibition of angiotensin converting enzyme (ACE), expel intestinal worm and softening or soothing the skin.

The vegetable is sold in the whole as well as in the sliced form. The intact vegetable can be stored for several days. However, the large size of the vegetable creates problem for its storage in supermarket and retail outlets, thus requiring some degree of preparation. Once cut, its quality deteriorates within a day or two. The high moisture content and presence of macronutrients such as sugars make it vulnerable to spoilage by microorganisms limiting its marketability. Recently, researchers have examined shelf life extension of cut

ash gourd using a combination of chemical pretreatment and partial dehydration [107]. However, after the treatment, the product was not fresh. It was therefore of interest to determine the feasibility of using gamma irradiation for improving the shelf life of ready-to-cook ash gourd and assessing the effect of such a processing technique on its various physico-chemical properties.

1.5.2. Drumstick (*Moringa oleifera*)

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, and is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is also called as the horseradish, drumstick, benzolive, kelor, marango, mlonge, moonga, mulangay, saijan, sajna or Ben oil tree. It is a slender softwood tree that branches freely, and can be extremely fast growing. Although it can reach heights in excess of 10 m (33 ft), it is generally considered a small- to medium-size tree. The fruits are tri-lobed capsules, and are frequently referred to as "pods." Commercial production of immature pods for processing is a large industry in India with about 1.2 million MT (metric tons) produced annually on 38,000 ha.

It is an important food commodity which has received enormous attention as the 'natural nutrition of the tropics'. Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Almost every part of the plant - the leaves, fruit, flowers and pods are used as a highly nutritive vegetable in many countries including India, Pakistan, Philippines, Hawaii and many parts of Africa [108]. The proximate composition of drumstick pods is shown in Table 13. The high concentrations of ascorbic

acid, oestrogenic compounds and β-sitosterol, iron, calcium, phosphorus, copper, vitamins A, B and C, α-tocopherol, riboflavin, nicotinic acid, folic acid, pyridoxine, β-carotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine present in drumstick leaves and pods make it a virtually ideal dietary supplement [109].

Apart from nutritional values, it has numerous medicinal uses, which have long been recognized in the Ayurvedic and Unani systems of medicine [110]. The various medicinal and pharmacological activities of different parts of the plant are summarized in Table 14. Almost all parts of this plant : root, stem, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders [111, 112].The medicinal properties of the plant have been attributed to the presence of bioactive phytochemicals including phenolic compounds, and a unique group of compounds called glucosinolates and isothiocyanates [113, 58]. They have been reported to possess hypotensive, anticancer, and antibacterial activity. The compounds responsible for these properties include 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate [1], 4-(α-L-rhamnopyranosyloxy)benzyl isothiocyanate [2], niazimicin [3], pterygospermin [4], benzyl isothiocyanate [5], and 4-(α-L-rhamnopyranosyloxy)benzyl glucosinolate [6] (Figure 8). While these compounds are relatively unique to the Moringa family, it is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or pro-vitamin A).

Table 13. Proximate composition and minerals and vitamins (content per 100 g of edible portion) in drumstick pods

		Content (per 100 g of edible portion)
Proximate composition	Moisture	86.9 %
	Protein	2.5 %
	Carbohydrate	3.7 %
	Fiber	4.8 %
	Fat	0.1 %
	Calcium	30 mg
	Phosphorous	110 mg
	Iron	120 mg
Vitamins	Vitamin C	14.1 mg
	Thiamin (B1)	0.05 mg
	Riboflavin (B2)	0.07 mg
	Niacin (B3)	0.62 mg
	Pantothenic acid (B5)	0.79 mg
	Vitamin B6	0.12 mg
	Folate (B9)	0.044 µg
Minerals	Sodium (Na)	42 mg
	Potassium (K)	461 mg
	Calcium (Ca)	30 mg
	Iron (Fe)	0.36 mg
	Magnesium	45 mg
	Manganese	0.26 mg
	Phosphorous	50 mg
	Zinc	0.45 mg

Table 14. Medicinal and pharmacological activities of different parts of drumstick tree

Plant part	Medicinal uses
Root	Antifertility, anti-inflammatory, stimulant in paralytic afflictions; act as a cardiac/circulatory tonic, used as a laxative, abortifacient, treating rheumatism, inflammations, articular pains
Leaves	Purgative, applied as poultice to sores, headache, used for piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh
Stem bark	Rubefacient, vesicant and used to cure eye diseases, prevent enlargement of the spleen and formation of tuberculous glands of the neck, to destroy tumors and to heal ulcers.
Gum	Used for dental caries, mixture of gum with sesame oil, is used to relieve headaches, fevers, intestinal complaints, dysentery, asthma and to treat syphilis and rheumatism
Flower	Aphrodisiac, abortifacient, cholagogue; anti-inflammatory, curing muscle diseases, hysteria, tumors, and enlargement of the spleen
Seeds	Seed extract exerts its protective effect by decreasing liver lipid peroxides, antihypertensive compounds thiocarbamate and isothiocyanate glycosids have been isolated from the acetate phase of the ethanolic extract of Moringa pods

It has been estimated that about one-fourth of all *Moringa oleifera* produce harvested is spoiled before consumption. Therefore, there are increasing interests to preserve the vegetable due to its medicinal and therapeutic properties [114]. Studies have been attempted to preserve the shelf-life of leaves and pods of this vegetable using various preservation techniques [115]. Treatments with germicide, salt and turmeric were employed in various combinations and it was followed with dehydration by sun drying, oven drying or wind drying. Microbiological and physico-chemical analyses were performed on the samples for a storage period of 4 months. The employed preservation

techniques involving dehydration were found to effectively extend the shelf life of *Moringa oleifera* without alteration in the nutritional value. However, no reports exist on the preservation or shelf-life extension of fresh-cut drumstick pods. It was therefore of interest to study the effect of gamma irradiation on the shelf-life and quality attributes of the fresh-cut drumstick.

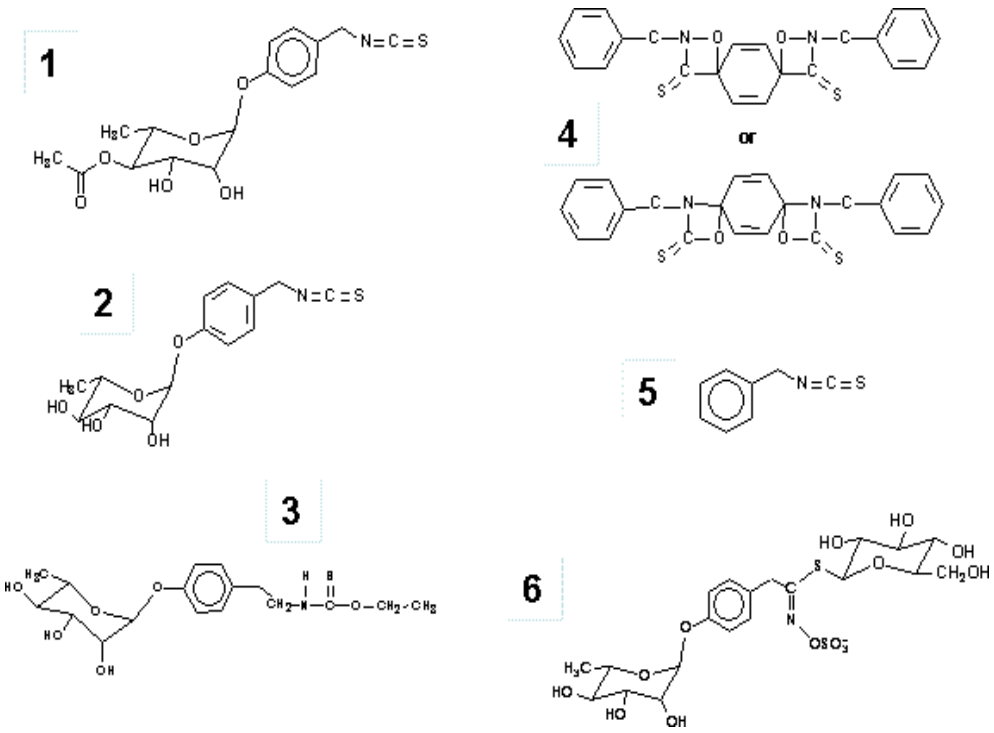


Figure 8. Glucosinolates reported in drumstick (*Moringa Oleifera*). 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate [1], 4-(α-L-rhamnopyranosyloxy)benzyl isothiocyanate [2], niazimicin [3], pterygospermin [4], benzyl isothiocyanate [5], and 4-(α-L-rhamnopyranosyloxy)benzyl glucosinolate [6]

1.5.3. Pumpkin (*Cucurbita pepo*)

Pumpkin (*Cucurbita pepo*) belongs to the Cucurbitaceae family which includes melons, squashes, cucumbers and gourds. It is defined botanically as a fruit although commonly regarded as a vegetable by the consumer. The pumpkin varies greatly in shape, ranging from oblate to oblong, with smooth, slightly ribbed skin and deep yellow to orange coloration. It is widely grown and consumed in many countries around the world [116] including India. The biggest international producers of pumpkins include the United States, Canada, Mexico, India, and China. According to estimations by the Food and Agriculture Organization of the United Nations (FAO), world production of pumpkins in 2007 was over 20 million tons, especially in China, India, Russia, United States, and Egypt [117].

In recent years, pumpkin has received considerable attention because of the nutritional and health protective value. The vegetable significantly contributes to nutrition as it is a rich source of minerals. It also contains a high content of carotenoids in its flesh, including β-carotene, lutein, and violaxantine. It also possesses several phytoconstituents belonging to the class of alkaloids, flavonoids, and fatty acids such as palmitic, oleic and linoleic acids [118]. In addition, many cultivars of pumpkin are characterized by a high content of vitamin C [118]. It possesses low calorific value (15–25 kcal in 100 g) and due to the presence of numerous, easily digestible nutrients it has become an important component of slimming diets. It regulates metabolism, lowers glucose level in blood, and possesses detoxicating as well as dehydrating properties. Other uses attributed to pumpkin species include defense against cancer [119] and diabetes and for internal as well as

external management of worms and parasites. Tables 15 and 16 represent the proximate composition and medicinal properties of the pumpkin.

Due to the large size of the vegetable it is difficult to store thus restricting marketability. Although, the intact vegetable can be stored for several days; once cut, it is highly perishable and deteriorates within 1-2 days. The high moisture content and presence of macronutrients such as sugars make it vulnerable to spoilage by microorganisms limiting its marketability. Recently, the shelf-life of cut pumpkin under modified atmospheric packaging (30% CO_2/ 70% N_2; 100% CO_2 and vacuum) and stored at refrigeration temperature was examined [120]. 100% CO_2 was inferred to be the best by these workers for a storage period of 5 days, with total microbial counts of 7.7×10^3 CFU g^{-1} and yeast and mould counts of approx 10 CFU g^{-1} after 5 days. However, to the best of our knowledge, efficacy of radiation processing in enhancing the shelf-life and quality of fresh-cut pumpkin has not been investigated so far.

Table 15. Proximate and minerals and vitamins content in pumpkin pulp (per 100 g of edible portion)

		Values per 100g of edible portion
Proximate composition	Moisture	94.2 %
	Protein	1.00
	Carbohydrate	4.04 %
	Fiber	1.9 %
	Fat	0.1 %
Vitamins	Vitamin C	9 mg
	Thiamin (B1)	0.05 mg
	Riboflavin (B2)	0.11 mg
	Niacin (B3)	0.6 mg
	Pantothenic acid (B5)	0.298 mg
	Vitamin B6	0.061 mg
	Folate (B9)	16 µg
	Vitamin E	0.44 mg
	Vitamin k	1.1 µg
Trace elements	Phosphorous	44 mg
	Iron	0.8 mg
	Calcium	21 mg
	Magnesium	12 mg
	Manganese	0.125 mg
	Potassium	340 mg
	Sodium	1 mg
	Zinc	0.32 mg

(Table adapted from USDA Nutrient Database)

Table 16. Medicinal and pharmacological activities of pumpkin

Anti-inflammoatory	Pumpkin seeds have anti-inflammatory properties that are very useful against the arthritis and joint inflammation.
Asthma	The anti-oxidants effectively protect the respiratory system from infections and free-radical attacks, reducing and healing asthma attacks.
Cholesterol	Pumpkin has high amounts of phytosterols that is similar to our human cholesterol. It can replace and normalize the cholesterol to a healthy level.
Depression	One of the cause of depression is the lack of trytophan in our diet. Pumpkin is rich with L-tryptophan, an essential amino acid that our body cannot manufacture. When this chemical compound is supplied, it activates the feeling of happiness and well-being, reducing the depressed mood.
Prostate cancer	The high content of zinc and carotenoids in pumpkin and its seeds help protect against prostate cancer. These compounds prevent enlargement of the prostate and over-stimulation of the male hormones that cause prostate problems.
Peptic ulcers	Pumpkin juice has medicinal properties that are calming to the gastrointestinal tract, healing to digestive conditions and peptic ulcers.
Kidney stones	Taking about 5 – 10 grams of pumpkin seeds daily prevents stones formation in the kidneys.

1.6. Scope of the work - Aims and objectives

Importance of convenience foods has increased considerably in recent times. These include fresh-cut ready-to-eat (RTE) fruits and ready-to-cook (RTC) vegetables. However, they have very limited marketing due to short shelf life. Gamma irradiation has been demonstrated as a promising tool for improving the shelf-life of such fresh-cut produce. Radiation processing is reported to act as an abiotic stress for the living tissues such as fresh-cut fruits and vegetables, thereby resulting in changes in the post harvest physiology of the product. Consumers and food safety advocates are therefore worried about the nutritional and chemical quality of such radiation treated produce.

One of the main mechanisms by which plants protect themselves against adverse environmental abiotic and biotic stress is production of reactive oxygen species (ROS). However, emerging data show that ROS production in certain situation can also contribute to the improved physiology and increased fitness of plants. The effect of different postharvest abiotic stresses (i.e., wounding, UV-light, hyperoxia, and the exogenous application of certain compounds or hormones) on the accumulation of phenolic compounds in fruits and vegetables has been evaluated in several studies. Nevertheless, little is known on the physiological basis for the accumulation of phytochemicals/antioxidants as a result of radiation processing in vegetables of Indian origin. Increasing the scientific knowledge in this area is critical to envisage strategies that permit the effective use of crops as bio-factories of nutraceuticals.

Vegetables during processing such as cutting and cooking give rise to characteristic aroma to the cuisine. Presence of glycosidic precursors of volatile aroma compounds has

been well established in the plant kingdom. These precursors are known to undergo hydrolytic or enzymatic breakdown during processing, resulting in the release of volatile aroma compounds, and hence enhanced aroma quality. The same hypothesis may be extended to explain the released aroma during processing in vegetables, however, it needs to be further explored. Few reports exist on the nature of aroma compounds present in most of the vegetables, especially of Indian origin. Radiation processing is also reported to result in breakdown of aroma glycoconjugates resulting in liberation of free aroma and thus an enhancement in overall flavor quality. No studies exist on the effect of irradiation on flavor precursors in majority of the vegetables. As aroma quality plays a critical role in deciding the consumer acceptance of a food product. The effect of radiation processing on the aroma constituents of fresh-cut produce thus needs to be investigated.

There is an increasing interest in the bioactive components of native plants and identifying their role as functional foods. This has resulted in a shift towards identifying native plant foods possessing novel organic molecules with unique bioactivities. In this context use of Indian vegetables as medicinal ingredients in Ayurveda has been in practice since early times. Despite being a treasure house of active constituents, very few studies exist on the nature of the constituents present in majority of the Indian vegetables.

Although, several reports exist in the literature regarding chemical composition, aroma and medicinal attributes of the above mentioned vegetables, extensive work is required to study the effect of gamma irradiation on various phyto-chemical aspects of these vegetables in order to understand the sensory and nutritional status of the radiation processed product.

Therefore, the major objectives of the thesis are as follows:

- To study the effect of gamma irradiation on the shelf life of ready-to-cook (RTC) products of selected Indian vegetables namely ash gourd (*Benincasa hispida*), pumpkin (*Cucurbita pepo*) and drumstick (*Moringa oleifera).*
- Physico-chemical characterization of the developed shelf stable radiation processed RTC products.
- Isolation and identification of aroma compounds, key odorants in the above mentioned vegetables and to study the effect of radiation processing and storage on the aroma profiles.
- To study the effect of radiation processing on some bioactive principles from the selected vegetables.

CHAPTER 2

MATERIALS AND METHODS

2. 1. Materials

2.1.1 Plant material

Vegetables - ash gourd (*Benincasa hispida*), bottle gourd (*Lagenaria siceraria*), pumpkin (*Cucurbita pepo*), lady finger (*Abelmoschus esculentus*) and drumstick (*Moringa oleifera*) were procured from a local grower around Mumbai, India. The vegetables were brought to the laboratory within 12 h after harvesting and immediately stored at refrigerated temperatures (10 ± 1 °C) before processing.

2.1.2. Chemicals and materials

Amberlite XAD-2 resin was procured from Sigma-Aldrich, USA. Reverse phase (C18) solid phase extraction cartridges with 6 mL volume and 0.5 g active phase were procured from Supelco, USA. Solid phase micro extraction (SPME) fibres made up of Poly dimethoxy siloxane-divinylbenzene-carboxen (PDMS/DVB/CAR) with film thickness of 0.65 μM and 1 cm length, were bought from Supleco, USA.

Methanol and dichloromethane was procured from Merck India Pvt. Ltd. Diethyl ether, n-hexane and n-butanol was purchased from S.D. Fine Chemicals, India. All solvents used were of analytical grade and were redistilled before use. HPLC grade solvents such as acetonitrile, methanol, glacial acetic acid and o-phosphoric acid were procured from Merck Ltd., Germany.

PCA, PDA, Murashige & Skoog Medium, (With Vitamins; without $CaCl_2$, Sucrose, IAA, Kinetin and Agar) and DPPH was purchased from HiMedia Laboratories, India. Folins-ciocalteu reagent was purchased from Merck, India. Boric acid and disodium hydrogen-*o*-phosphate were from Qualigens Fine Chemicals and sodium dihydrogen-*o*-

phosphate was procured from Thomas Baker Ltd. Potassium ferricyanide ($K_3[Fe(CN)_6]$) and sodium bicarbonate ($NaHCO_3$) were from BDH Laboratory Chemicals and Chemco Fine Chemicals, respectively. Hydrogen peroxide and *m*-phosphoric acid were procured from S.D. Fine Chemicals Ltd.

Various standard compounds used i.e. acetoin, acetobromo-D-glucose, phenolic acids, carotenoids, enzyme preparations – pectinase (≥ 5 U/mg protein), myrosinase (≥ 100 U/g) and sulfatase (≥ 10000 U/g) were bought from Sigma Aldrich, USA.

2.2. Methodology

2.2.1. Development of ready-to-cook (RTC) vegetables using radiation processing

2.2.1.1. Preparation of RTC vegetables

Whole ash gourd, bottle gourd, pumpkin, lady finger and drumsticks were washed in running tap water to remove adhered dust. After washing, ash gourd, bottle gourd and pumpkin were hand peeled and cut into small pieces of dimensions 2.5 cm × 2.5 cm × 0.7 cm with a sharp sterile knife. The lady fingers and drumstick pods were transversely cut in to pieces of length 1.5 and 4.0 cm, respectively. The cut pieces of each vegetable were separately randomized before packaging, and were packed (100 g) into polystyrene trays (inner dimensions: 9 cm × 9 cm × 2.5 cm). The trays were then over-wrapped all around with cling film (Flexo film wraps ltd., Aurangabad, Maharashtra, India) and sealed to avoid any leakage. Film used in the present study had thickness of 8–10 µm and permeability to oxygen and carbon dioxide as 1.7 and 10.3 cm^3m^{-2} s^{-1} Pa^{-1} respectively (data as provided by supplier).

2.2.1.2. Radiation processing and storage

Packaged cut vegetables were subjected to various radiation doses (0.5, 1.0, 1.5, 2.0 and 2.5 kGy) in a cobalt-60 irradiator (GC-5000, BRIT, Mumbai, India) having a dose rate of 1.64 Gy/s. Irradiator was calibrated by Fricke dosimeter before start of experiment and had a dose uniformity ratio of 1.2. Irradiated samples were stored in the dark at 10 ± 1 °C. In preliminary studies on RTC products carried out in our laboratory, the optimal storage temperature was found to be 10 °C. Storage at a lower temperature (4 °C) induced chilling injury resulting in low aroma scores whereas at a higher temperature of 15 °C, physiological as well as microbial spoilage was very rapid resulting in shorter shelf life. Non-irradiated samples of respective vegetables stored at 10 ± 1 °C were used as controls during the entire storage period. Three replicates were prepared for each dose and storage day. The samples were examined at different time intervals during storage. During initial investigations, it was observed that cut bottle gourd developed browning immediately after cutting. In radiation processed cut lady finger, the sticky mucilaginous secretions increased with radiation dose, thus making irradiated lady finger unacceptable by the sensory panel. The treatment of anti-browning reagents in case of bottle gourd and some chemical treatments in case of lady finger prior to irradiation could help in their shelf-life improvement. It was therefore inferred that radiation processed bottle gourd and lady finger were not amenable for development of RTC products and hence they were not studied further.

2.2.1.3. Optimization of quality parameters

Experimental design and subsequent analysis of results were performed by a software Design Expert 8.0 (State-ease Inc., U.S.A.). Control and radiation-treated samples were analysed on day 0, 5, 8, 12 and 15 after packaging for ash gourd and drumstick, whereas for pumpkin, the analyses were conducted on days 0, 7, 14, 21 and 28 after packaging. A full factorial experimental design was employed with gamma radiation dose and storage time as variable factors to be optimized [121]. Experiments were planned at different radiation doses (0, 0.5, 1.0, 1.5, 2.0 and 2.5 kGy). Three replicates were prepared for each dose and storage day. Parameters studied for analyzing product quality were microbial load, color, texture and sensory acceptability.

Models for individual responses were generated by fitting experimental data into third order polynomial equations and then removing insignificant terms by backward regression method as reported earlier [122]. In this approach, model terms with highest values of partial probability (p-value) are removed first and the process is stopped when the p-value of next term out satisfies the specified alpha out criterion. Value of alpha out was kept 0.1 which leads to an overall model with terms significant at 0.05 levels. The models were analyzed by analysis of variance (ANOVA) and the coefficient of determination (R^2) was used to quantify the predictive capability of the model. Fitted polynomial equations were then plotted in order to visualize the interrelationship of response and experimental levels of each factor. Optimal conditions were then derived using numerical optimization process. Criteria for desired microbial and sensory

qualities were provided and model equations solved to obtain optimum processing conditions.

2.2.1.3.1. Microbial quality

Standard methods were used to enumerate total microbial load present in the RTC vegetables at each sampling time and treatment for the intended duration of storage [123]. Enumeration of total aerobic mesophilic bacteria was carried out by pour-plate method on plate count agar (Himedia, Mumbai, India). Incubation was done at 37 °C for 24 h. Total yeast and mould count was performed by pour-plate method on potato dextrose agar (Himedia, Mumbai, India) supplemented with 0.01 g L^{-1} tartaric acid to lower pH of the medium to 3.5. Plates were incubated at 37 °C for 48 h. Microbial counts were expressed as $\log_{10}$ CFU g^{-1}. Each analysis was performed in triplicate.

2.2.1.3.2. Sensory quality

Cut pieces of each vegetable were cooked separately in boiling water for 5 min and presented to the assessors in white trays for sensory assessment in different sessions. Hedonic testing was carried out for each vegetable by 15 panelists of trained sensory panel using a 9-point scale with 1, dislike extremely or not characteristic of the product and 9, like extremely or very characteristic of the product [123]. Parameters evaluated were colour, aroma, texture, taste, aftertaste and overall acceptability. Irradiated and stored samples were compared with fresh-cut control samples of the respective vegetables on each day of study to analyze all the parameters.

Color and firmness were also assessed instrumentally for all the samples prepared. Surface colour values were evaluated using a colorimeter (CM- 3600d Konica Minolta sensing Inc., Japan) by measuring the three Commission Internationale de l'Eclairage (CIE) coordinates, L (lightness), a (−green, +red), and b (−blue, +yellow). The instrument was calibrated using a white tile supplied along with the equipment. Six pieces of each vegetable were selected randomly from every packaged tray for color measurement and results represent their average. Firmness was measured using a texture analyzer (TA.XTPLUS, Stable Microsystems Ltd., Surrey, England) with a 980 N load cell, equipped with a cone probe (P/45C s/s batch No. 3889) for ash gourd and pumpkin, while a needle probe (P/2N s/s batch No. 10451) was employed for piercing test through the drumstick samples. The test was designed with a trigger force of 0.147 N and test speed of 0.5 mm/s. The peak force required to penetrate samples was referred as a measure of firmness of the sample. Data were collected for six replicates from each tray and results were expressed as their average.

At optimized processing conditions, sensory evaluation was also performed by quantitative descriptive analysis (QDA) [124] along with hedonic analysis. A trained panel consisting of 10 members, 6 males and 4 females assessed the samples using unstructured 150-mm scale. The sensory attributes for each of the vegetable studied were finalized during prior training sessions conducted. The sensory attributes assessed for pumpkin were color (yellow), aroma (pumpkin-like, irradiated and musty), and taste (pumpkin like and irradiated); for ash gourd the attributes were color (green), texture, aroma (ash gourd-like, buttery irradiated and musty), and taste (ash gourd like and

irradiated); and that for drumstick were color (green, brown), texture, aroma (drumstick-like, green, irradiated and musty), and taste (sweet, drumstick like and irradiated). Assessments were repeated twice and sensory data were collected by measuring distance (mm) from the origin. Non-irradiated ash gourd samples exhibited visible browning after a storage period of 5 d, while browning was inhibited in the radiation processed samples throughout the intended storage.

2.2.2. Characterization of the developed products at optimized parameters

2.2.2.1. Nutritional quality

2.2.2.1.1. Preparation of methanolic extract

Each vegetable (10 g) was separately homogenized and extracted twice with 20 mL distilled methanol in an omnimixer (Sorvall, U.S.A). The extract was centrifuged at 3000 x g for 30 min and the supernatant thus collected was made to a final volume of 50 mL by adding methanol. Aliquots were used for determining antioxidant status in terms of total phenolics and DPPH radical scavenging potential.

2.2.2.1.2. Radical scavenging activity

DPPH radical scavenging assay was used to evaluate total antioxidant activity of the RTC vegetables according to the procedure of Wen et al. [125]. The final reaction mixture used for the study was 3 mL (25 µL methanolic extract + 1 mLof DPPH solution (200 µM in methanol) + 1975 µL methanol). After incubation under dark conditions for 30 min, absorbance was measured at 516 nm. Total antioxidant activity was expressed as the

Gallic acid equivalent mass per mass of the vegetable studied (mg kg^{-1}). [GAE (mg kg^{-1}) = (((% Inhibition – 3.866)/15.67) X 10 X 40)/10); R^2 = 0.97]

2.2.2.1.3. Total phenolic content

Total phenolic content was evaluated in accordance with the Folin–Ciocalteu procedure [126]. Part of the methanolic extract obtained as above (Section 2.2.8.1) was treated with polyvinyl polypyrrolidone (10 gL^{-1}, PVPP, Sigma–Aldrich, USA) to remove phenolic compounds from extract. PVPP was purified as per the procedure detailed earlier [127]. The mixture was then incubated overnight in an orbital shaker at 25 °C at 2.5 oscillations per s. PVPP was then removed by centrifugation at 21,000 × g for 10 min at 4 °C. The supernatant was collected and the sediment (PVPP–polyphenol complex) was discarded. The absorption of the supernatant and original extract was measured at 725 nm using UV–visible spectrometer (Helios α, Thermofisher Scientific, USA) in accordance with the Folin–Ciocalteu procedure. The content of total phenolics in the vegetable was determined by the difference between phenolic content obtained before and after PVPP treatment and then expressed as the Gallic acid equivalent mass per mass of the fresh vegetable as mg kg^{-1}. [GAE (mg kg^{-1}) = ((O.D + 0.017)/0.048); R^2 = 0.99]

2.2.2.1.4. Ascorbic acid content

Total vitamin C content of the processed vegetables was estimated in accordance with standard AOAC official microfluorometric method [128]. Cut vegetables (20 g) were extracted separately with 20 mL of freshly prepared extracting solution containing metaphosphoric acid (0.3 mol L^{-1}) and acetic acid (1.4 mol L^{-1}). Homogenate was then centrifuged at 16,000 × g for 15 min at 4 °C and the supernatant was treated with

activated charcoal (20 g L^{-1}) with vigorous shaking to convert ascorbic acid into dehydroascorbic acid. The mixture was again centrifuged at 21,000 × g for 10 min at 4 °C to remove charcoal. Aliquots of this supernatant (500 µL) were added to a solution containing equal volumes of boric acid and sodium acetate (3% boric acid in 3.67 mol L^{-1} sodium acetate solution). The solution was allowed to stand for 15 min and the total volume was then adjusted to 10 mL using milli Q water. This was designated as blank solution. To prepare the sample solution, another aliquot of 500 µL was mixed with an equal volume of sodium acetate solution (3.67 mol L^{-1}) and the total volume was adjusted to 10 mL using milli Q water. A 0.4 mL aliquot of both (sample and blank) solutions was separately treated with 1 mL of *o*-phenylenediamine solution (0.02 %) (Sigma Chemical Co. Ltd., St. Louis, USA), vortexed and incubated for 35 min at ambient temperature (25 ± 2 °C). Ascorbic acid reacts with *o*-phenylenediamine to form a fluorescent conjugate. Total conjugate formed was measured (EX_λ 350 nm; EM_λ 430 nm, Bandwidth 5 nm) using CS-5000 fluorimeter (Shimadzu Corporation, Japan). The same process was followed for standard ascorbic acid solutions of known concentration (0.1–0.0015 %) to obtain a standard curve. Linear regression was then used to determine the concentration of ascorbic acid in each sample. The content of vitamin C per mass of ash gourd was expressed in mg kg^{-1}. All analyses were carried with three independent samples each analyzed in triplicate.

2.2.2.2. Aroma quality

2.2.2.2.1. Isolation and identification of free aroma compounds

2.2.2.2.1.1. Isolation of free aroma

Optimization of procedures for isolation of free aroma was carried out for the selected vegetables (ash gourd, pumpkin and drumstick). Free aroma volatiles were isolated using three different techniques – simultaneous distillation extraction (SDE), high vacuum distillation (HVD) and solid phase microextraction (SPME). Extraction using SDE and HVD was essentially performed as per procedure described earlier [129, 130] while procedure followed for SPME extraction was as per Sagratini et al. [131]. A flow diagram depicting different methodologies followed for extraction of free aroma volatiles is demonstrated in Figure 9.

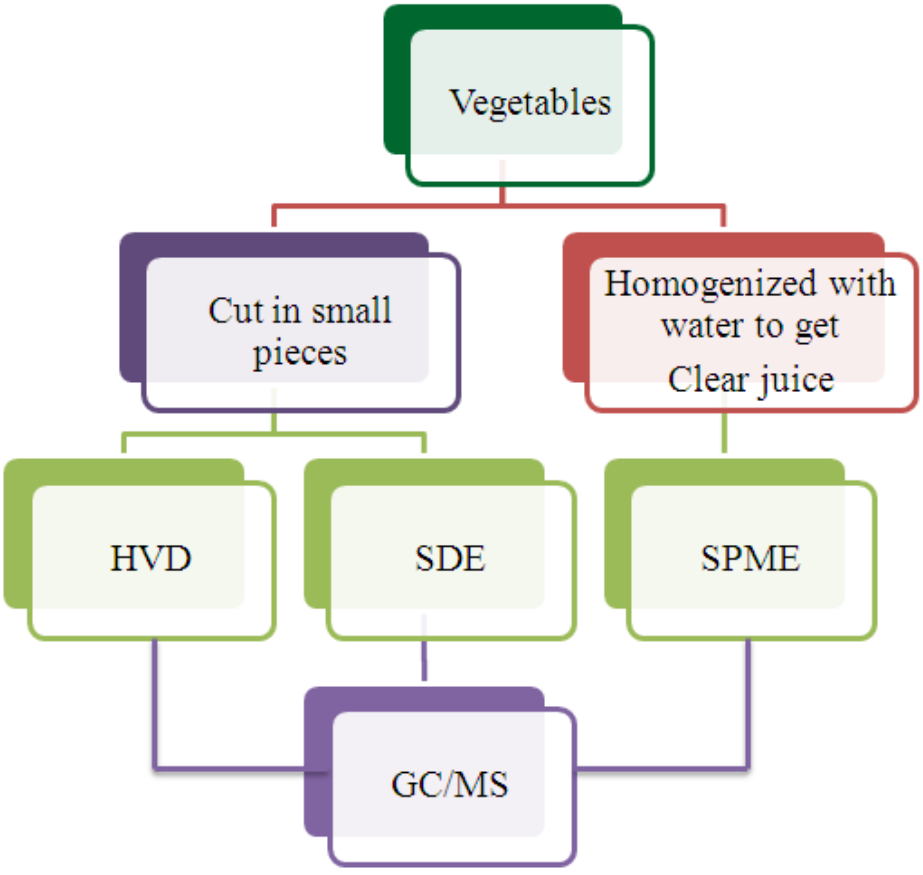


Figure 9. A schematic representation of the different methodologies adopted for the isolation and analysis of free aroma volatiles.

A brief decription of the main methodologies adopted for aroma isolation is provided below.

A) Simultaneous Distillation Extraction (SDE): Ash gourd, pumpkin and drumstick, 200 g each was cut into small pieces and separately subjected to SDE [132] for 2 h using peroxide free diethyl ether (AR Grade, S.D. Fine-Chem. Ltd., Mumbai, India) as extracting solvent. The solvent was then removed by passing a slow stream of nitrogen to obtain the volatile oil.

B) Solid Phase Microextraction (SPME): To each cut vegetable sample (50 g) was added 10g NaCl and 10 mL distilled water and then homogenized (B400, Buchi, Switzerland) while maintaining chilled conditions. The homogenate obtained was filtered through double layered washed & dried muslin cloth. The extract obtained was centrifuged at 12,000 rpm at 20 °C for 15 min. To the sample, 2-octanol (1.25 µg) was further added as internal standard. Samples were equilibrated for 30 min at 30 °C with continuous stirring and headspace was subsequently extracted by preconditioned PDMS/DVB/CAR fibres (Supleco, USA) for 20 min at 30 °C. After extraction, fibres were desorbed (270 °C) in the injection port of the GC/MS equipment. Quantification was performed by comparing peak areas of compounds with that of internal standard and results obtained as µg kg^{-1}.

C) High vacuum distillation (HVD): High vacuum distillation was carried out according to the procedure reported in literature [129, 130]. Cut ash gourd (200 g) was frozen in liquid nitrogen before use. The sample was placed in a glass tube (5 cm i.d., 25 cm length) and then connected to a distillation unit maintained under vacuum (1 x 10^{-3}

torr). The distillate collected in a receiving tube (3 cm i.d., 20 cm length) was maintained at low temperature using liquid nitrogen. The isolate (50 ml) was extracted with diethyl ether (3 x 5 ml). Solvent was removed by a slow stream of nitrogen to obtain oil that was subjected to GC/MS analysis.

2.2.2.2.1.2. GC–MS analysis of free aroma compounds

The aroma concentrates obtained from SDE, HVD and SPME were subjected to GC–MS analysis on a Shimadzu GC–MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a GC-17A gas chromatograph and provided with a DB-5 (J&W Scientific, California, USA) capillary column ((5 %-phenyl)-methylpolysiloxane, length, 30 m; i.d., 0.25 mm and film thickness, 0.25 µm). The operating conditions were: column temperature programmed from 60 to 200 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min. and further to 280 °C at the rate of 10 °C/min, held at final temperature for 20 min; Injector and interface temperatures, 210 and 280 °C, respectively; carrier gas helium (flow rate, 0.9 ml/min); ionization voltage, 70 eV; electron multiplier voltage, 1 kV. Peaks were tentatively identified by comparing their mass fragmentation pattern with that of standard compounds wherever available, from standard spectra available in the spectral library (Wiley/NIST Libraries) of the instrument as well as by comparing RI (retention index) values of the compounds with literature data. The content of the identified compounds in the essential oil obtained from SDE and HVD methods were estimated from a standard curve (R^2 = 0.99) of concentration versus peak area prepared using different concentrations of standard

acetoin (0.01–20 µg/µl) for all the three vegetables studied. The contents of various aroma compounds identified were expressed as µg/kg of the fresh weight of the vegetable.

2.2.2.2.2. Isolation and identification of bound aroma precursors

Optimization of procedures for isolation of bound aroma precursors was carried out for all the three selected vegetables. Aroma glycosides were isolated using XAD column and solid phase extraction (SPE) using C18 cartridges. Extraction using XAD column was essentially performed as per procedure described earlier by Arul et al. [133] while procedure followed for SPE extraction was as per Solis et al. [134]. A flow chart depicting different methodologies followed for extraction of bound flavour precursors is demonstrated in Figure 10**.**

The methodologies adopted for extraction of bound aroma glycosides are briefly described below:

2.2.2.2.2.1. Preparation of extracts

For isolation of bound aroma glycosides, vegetable extracts were prepared using two different approaches as described below:

A) Cut vegetables (50 g) were homogenized with 200 mL of methanol using a high speed mixer (Omnimixer, Sorvall, USA) for three min at a speed corresponding to the position of the knob at position four. The resultant slurry was filtered through a Buchner funnel under suction. Residue obtained was then further extracted twice using same solvent. Extracts from all three extractions were pooled and evaporated to dryness under vacuum (40 mbar, 40 °C) using a rotary evaporator (Buchi, Switzerland). Dried residue

was then dissolved in 150 mL of distilled water. This was designated as aqueous methanol extract.

B) In second approach, 150 g of cut vegetables were added to 50 ml of distilled water and then homogenized using a high speed homogenizer (B400, Buchi, Switzerland). Resulting slurry was then centrifuged (5810R, Eppendorf, Germany) at 12000 rpm for 15 min. Clear juice thus obtained was directly used for further extraction of aroma glycosides.

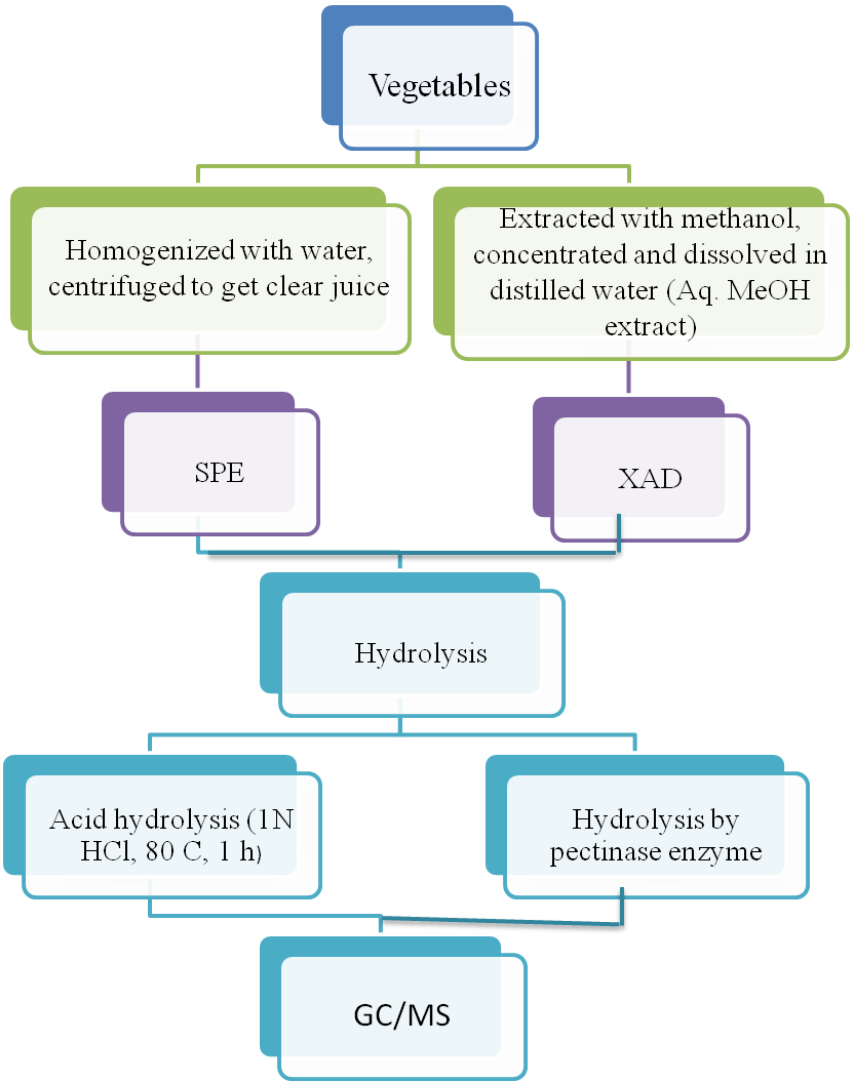


Figure 10. A schematic representation of the different procedures followed for isolation and analysis of bound aroma precursors.

2.2.2.2.2.2. Isolation of aroma glycosides

Bound aroma glycosides from the vegetables were extracted using two different extraction methods which are briefly described below:

A) XAD: Aqueous methanol extract of each of the vegetable was subjected separately to XAD column for isolation of aroma glycosides. XAD resin was packed in glass column of 2.5 cm I.D. up to a length of 18 cm using methanol. Column was then washed using 200 mL of methanol followed by 200 mL of diethyl ether. The column was finally equilibrated with 200 mL of distilled water. Vegetable extracts (200 mL) were separately loaded on XAD columns with a flow rate of 1.5 mL min^{-1}. Post loading, column was washed with 200 mL deionized water and diethyl ether to remove polar and mid-polar impurities. Subsequently, elution with 200 mL methanol yielded the bound aroma precursors or aroma glycosides. Methanol fraction was further evaporated to dryness using rotary evaporator and the residue obtained was finally dissolved in 10 mL of distilled water for further analysis.

B) Solid Phase Extraction (SPE): SPE cartridges were washed with 10 mL methanol and then further equilibrated by eluting with 10 mL of distilled water. Vegetable juice (15 mL) was then loaded on the cartridge while maintaining a flow rate of 1 mL min^{-1}. Cartridge was subsequently, washed again with 10 mL of distilled water followed by 10

mL of diethyl ether and the bound flavor precursors were then eluted with 10 mL of methanol.

2.2.2.2.2.3. Identification and quantification of bound aroma

The extracts obtained using XAD and SPE extraction were either acid hydrolyzed using 1 N HCl or subjected to enzymatic hydrolysis using pectinase (Figure 10). Detailed procedure followed for hydrolysis is as follows:

A) Acid hydrolysis: Extract containing aroma glycosides (10 mL) obtained from XAD as described in section 2.2.2.2.2.2 was made to 1 N with concentrated HCl. This solution was then heated at 80 °C for 1 h in a stoppered conical flask. The hydrolysate was extracted with diethyl ether (3 x 20 ml) and the organic layer containing free aglycones was repeatedly washed with distilled water till free of acid. The organic layer was dried over sodium sulphate and then concentrated to less than 1 mL volume using Kuderna-Danish concentrator (Supelco, USA). Extract was finally concentrated to a volume of 100 µL using a gentle stream of nitrogen and then injected (1 µL) into GC/MS equipment for identification of free forms.

B) Pectinase hydrolysis: Methanol fraction obtained from SPE containing aroma glycosides obtained as described in section 2.2.2.2.2.2 was concentrated to dryness using rotary evaporator and the residue was dissolved in 15 mL of citrate phosphate buffer (0.1 M, pH 5). Pectinase preparation (500 µL) was added into this solution and the samples were kept for incubation at 37 °C for 48 h. Sample hydrolyzed with pectinase was taken into a 40 mL SPME vial, 4.5 g of NaCl added and then equilibrated at 30 °C for 45 min. The free aroma compounds were extracted with a preconditioned

(270 °C, 10 min) SPME fibre (PDMS/DVB/CAR). Extraction was carried out by exposing SPME fiber in sample headspace for 20 min at 30 °C. Post extraction, fiber was desorbed in split/splitless port of GC/MS kept at 270 °C and the analysis was carried out in splitless mode. Before SPME extraction, 2-octanol (1.25 µg) was added as internal standard. Each analysis was carried out in triplicate for quantification.

Samples were analyzed on a GC/MS (QP5050A, Shimadzu, Japan) instrument equipped with RTX-5 column (5% diphenyl-dimethyl-polysiloxane, 0.25 µm I.D., 30 m length, Restek corporation, USA). The conditions for analysis were similar as described in section 2.10.1.2. Data was acquired in scan mode from m/z 40 to 350. Peaks were identified by comparing their mass fragmentation pattern and Kovat's indices with that of standard compounds as well as from the data available in the spectral (Wiley/NIST) libraries of the instrument. Quantification was performed by comparing peak areas of compounds with that of internal standard and results obtained as µg kg^{-1}.

2.2.2.2.3. Chemometric analysis of the aroma data

Data obtained for free as well as bound aroma (TIC v/s retention time) was exported in ascii format to Microsoft Excel using the inbuilt software of GC/MS. The corresponding ascii data were then separately subjected to PCA for analyzing changes as a result of radiation treatment and storage. Score plots were drawn and results were visually analyzed. Data obtained for principal components was further analyzed using ANOVA and means comparison was performed using Duncan's multiple range test. All the statistical analysis was carried out using XLSTAT 2012 software (Addinsoft Inc. U.S.A.).

2.2.2.2.4. Identification of aroma impact compounds

2.2.2.2.4.1. GC-O analysis

The aroma concentrates of the three vegetables obtained from SDE and in SPME mode were subjected to GC-O analysis on a Shimadzu GC-MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a GC-17A gas chromatograph, provided with a DB-5 (J&W Scientific, California, USA) ((5%-Phenyl)-methylpolysiloxane, length, 30 m; id., 0.25 mm and film thickness, 0.25 mm) and an olfactory detection port (ODP-2, Gerstel, Germany). A fixed splitter (1:1) was used at the end of capillary column. One part of the column flow was directed to mass detector in gas chromatograph system, while the other part was directed to an olfactory detection port (ODP). Humidified air was introduced into the sniff port up-stream at 100 mL/min near the point where the capillary column first entered the sniffing port. The air carried the capillary column effluent into a glass funnel where sensory analysis was done. The transfer line to the GC-O sniffing port was held at 280 °C. Helium was introduced as make up gas in the transfer line to the sniffing port at 8 mL/min. Simultaneous sniffing at the exit port of ODP and corresponding response in the mass detector allowed identification of the compound responsible for the perceived aroma based on its mass fragmentation. The operating conditions were similar to that provided in section 2.10.1.2. Peaks corresponding to various aroma notes sniffed by the assessors were identified by comparing their mass fragmentation pattern with that of standard spectra available in the spectral library (Wiley/NIST Libraries) of the instrument as well as based on their

retention indices on the DB-5 column previously reported, odor quality and mass spectral data with those of standard compounds wherever available.

2.2.2.2.4.2. Global Olfactometric analysis and determination of odor activity values (OAV)

Analysis was carried according to the conditions reported by Guen et al. [135]. A panel of 9 judges (4 females and 5 males, aged 25-50 years) trained in odor recognition and experienced in GC-O was selected. The testing room was at 24 ± 1 °C and 50 ± 5% RH; the illumination was a combination of natural and non-natural (fluorescent) light. Panelists assigned odor properties to each note detected. The number of times a note was perceived by sensory panel was defined as its detection frequency. Detection of odor by fewer than 4 panelists was considered as noise [136]. Sniffing was divided into two parts each of 15 min. Panelists were divided in two batches consisting of four and five people. Panelists involved in first 15 min were asked to sniff another half of the sniffing in next round. The second batch sniffed the remaining 15 min of the first round. In the second round of analysis, first batch of panelists analyzed the aroma notes eluting from 15 to 30 min while the second batch analyzed the aroma compounds eluting during 0-15 min. Thus, each person participated in the sniffing of both parts but during two distinct sessions to avoid tiredness. A final aromagram of detection frequency (DF) versus RI was obtained after summing up all the nine individual aromagrams.

To evaluate the contribution of a chemical compound to the overall aroma of a vegetable the odor activity value (OAV) was determined. OAV is a measure of importance of a specific compound to the odor of a sample. It was calculated as the ratio

between the concentration of an individual compound and the perception threshold as reported in literature [137].

2.2.2.3. Headspace gas composition inside packages

Oxygen concentration in package headspace was measured using a gas chromatograph (GC 2010, Shimadzu Corporation, Japan). The GC was equipped with split/splitless injector, a molecular sieve column (length 30 m, 0.35 I.D., RT-Msieve 5A, Restek Corporation, USA) and a TCD detector. Injection port temperature was 35 °C. Initial column temperature was kept 30 °C for 5 min and then raised at rate of 0.167 °C/s to 100 °C. The column was further held at 100 °C for 5 min with the TCD current and temperature maintained at 90 mA and 110 °C, respectively. Sampling was done by inserting a hypodermic needle into the bag through an adhesive septum, previously stuck to the bag. A 0.1 mL of headspace sample was extracted and injected into a split ratio of 5. Only O_2 and N_2 could be evaluated on the column used in the study. Based on observed O_2 and N_2 concentrations in the package headspace, actual concentrations of O_2 and CO_2 (% O_2 and % CO_2) were calculated using following equations:

$$\%\ O_2 = \frac{(\text{Observed \% } O_2)}{(\text{Observed\% } N_2)} \times 78.084 \qquad (1)$$

$$\%\ CO_2 = 100 - \frac{(\text{Observed \% } O_2)}{(\text{Observed \% } N_2)} \times 78.084 + 78.084 \qquad (2)$$

(The atmospheric composition of N_2 was taken as 78.084%).

Analysis was done in triplicates using three independent sample trays on each day.

2.2.2.4. Gamma irradiation induced browning inhibition

2.2.2.4.1. Isolation and identification of phenolic compounds

Isolation of phenolic compounds: Ash gourd (20 g) from each tray was extracted twice with 20 ml methanol. The extract was then centrifuged at 12000 rpm for 20 min and the supernatant collected. It was filtered through 0.22 µm membrane filters (Millipore) prior to analysis by HPLC.

HPLC analysis: Phenolic constituents were evaluated by a reversed-phase high-performance liquid chromatography (RP-HPLC, Jasco, Tokyo, Japan), equipped with a degasser, a quaternary pump delivery system, and a diode array detector. Sample (20 µL) as obtained above was loaded on to a C-18 column (250 mm × 4.6 mm length, 5 µm particle size) and eluted with mobile phase (flow rate 1 mL/min) consisting of solvent A- 1.5% *o*-phosphoric acid in Milli Q water and solvent B- acetonitrile: acetic acid: phosphoric acid: water (20:24:1.5:54.5). The gradient program used was as follows: 0 min- 80% A; 30 min- 33% A; 33min- 10% A; 40 min – 0% A. The column was conditioned with acetonitrile for 10 min after every run. The peaks were identified by comparing retention times and UV-Vis spectra in the 200-500 nm range with those of the corresponding authentic standards. Quantification of identified compounds was performed using calibration curves of the corresponding standard compounds at the specific absorption maximum. Co-chromatography with added authentic standards was also performed for further confirmation of the identified phenolic constituents.

2.2.2.4.2. Effect of radiation processing on enzymes involved in browning

Phenyl alanine lyase (PAL): PAL activity was measured according to the protocol reported by Degl'Innocenti et al with some modifications [138]. Enzyme extracts were obtained by blending 1:1 (w/w) cut ash gourd pieces with borate buffer (50 mM, pH 8.5)

containing 10 mM 2-mercaptoethanol and 0.5 g of PVPP under chilling conditions. The homogenate was filtered through 4 layers of cheesecloth and centrifuged at 21,000 x g at 4 °C for 20 min. The supernatant was used for subsequent assay. The reaction mixture consisting of 1 mL of 50 mM L-phenylalanine, 0.5 mL of the supernatant and 1.5 mL of borate buffer was incubated at 40 °C for 1 h. The absorbance was measured at 290 nm before and after incubation. Difference between the two gave the amount of product (cinnamic acid) formed. One unit of PAL activity equals the amount of PAL that produced 1 μmol of *trans*-cinnamic acid in 1 h and was expressed as μmol g^{-1} FW h^{-1}.

Peroxidase (POD): The enzyme extract was by blending 1:1 (w/w) cut ash gourd pieces with 0.05 M phosphate buffer at pH 7.0 under chilling conditions. Blending was performed in the presence of 10 % (w/w) polyvinylpyrrolidone (Sigma, St. Louis, MO, USA) to bind polyphenols. The extract was then centrifuged at 4 °C at 21,000 x g for 10 min. The supernatant was collected and used for peroxidase (POD) activity. Assay was carried according to the procedure reported by Degl'Innocenti et al (2005) with some modifications 138. The chlorogenic acid peroxidase assay contained 750 μL of 50 mM potassium phosphate buffer, pH 6.5; 100 μL of 80 mM chlorogenic acid; 50 μL of extract, and 100 μL of 35 mM H_2O_2. The caffeic acid peroxidase assay contained 750 μL of McIlvaine buffer (114 mM Na_2HPO_4 and 43 mM citric acid), pH 5.5; 100 μL of 80 mM caffeic acid, 50 μL of extract; and 100 μL of 35 mM H_2O_2. In all cases, POD assays were initiated by the addition of H_2O_2 (100 μL, 35 mM). Absorbance was measured at 410 nm for chlorogenic acid and 470 nm for caffeic acid peroxidase activity. The activities of PODs were expressed as A_λ min^{-1} g^{-1} fresh weight.

Polyphenol oxidase (PPO): Enzyme extraction procedure followed was same as that for POD assay as mentioned above. PPO activity was assayed spectrophotometrically (Model UV 4-100, Unicam, Cambridge, UK) at 25 °C according to methodology of Mishra et al. [139] based on the absorption at 420 nm of the brown polymers formed when catechol is oxidized in the presence of PPO. The reaction mixture consisted of 600 µL buffer, 200 µL enzyme extract and 200 µL substrate. The increase in absorbance at 420 nm was monitored at 1 min intervals for 10 min and the average change in absorbance per minute was calculated from the linear region of absorbance. One unit of enzyme activity was defined as the amount of enzyme which caused a change of 0.01 in absorbance/min. The PPO activity was expressed as U g^{-1} fresh weight.

PPO inhibition – Kinetic studies: The inhibitory activity of alpha resorcylic acid for ash gourd PPO was investigated. Various concentrations of catechol (25 - 250 mM) and alpha resorcylic acid (50 - 250 mM) were prepared in phosphate buffer (pH 7). Catechol solutions (150 µL) and alpha resorcylic acid (100 µL) were mixed together in a cuvette containing 550 µL of the buffer. The reaction was initiated by addition of 200 µL of PPO preparation. The readings were taken in the similar manner as described above (section 2.11.1.1.4.1(C)). The degree of inhibition was expressed as percentage inhibition ($I = 100(A^* - A)/A$, where A and A* were enzyme activities with or without inhibitor, respectively). Kinetic parameters of alpha resorcylic acid inhibition were determined using double-reciprocal plots (Lineweaver-Burk) of enzyme activity vs catechol concentration. I_{50} value of alpha resorcylic acid was determined from the plot

between enzyme activity vs inhibitor concentration, where I_{50} value is the mM concentration of the inhibitor required to inhibit enzyme activity by 50 %.

2.2.2.4.3. Scanning Electron Microscopic (SEM) analysis

Control and irradiated ash gourd pieces on day 12 of storage were subjected to SEM analysis using a facility (SEM Model Quanta 200 SEM, FEI Company, Oregon, USA) available from Icon Analytical Equipments Pvt. Ltd., Mumbai, India. The sample was fixed on a carbon tape and observed at different magnifications using a low vacuum mode at 65 Pa and 20 kV.

2.2.2.4.4. Electrolytic leaching

The electrolytic leaching was measured using the procedure as reported earlier. 10 g of cut ash gourd was taken in a beaker containing 50 ml of deionized water and stirred on a magnetic stirrer for 2 minutes. It was then filtered through Whatman filter paper and conductance of the filtrate was measured using a hand-held conductivity meter (HI 9812, Hanna Instruments, Italy). Spectrophotometer readings of the filtrate were also taken at 280, 320 and 420 nm for assessment of nature and content of compounds leaching out and hence attributing to the browning.

2.2.2.4.5. Statistical analysis

Statistical significance was assessed by two-way analysis of variance to determine the effect of radiation processing and storage time. Multiple means comparison was done by Duncan's test ($p<0.05$). For each measurement, three replicates of 3 independent samples were tested.

2.2.3. Isolation, identification and studying the effect of radiation processing on bioactive phytochemicals

(A) Ash gourd

2.2.3.1. Compounds with plant growth promoting activities

2.2.3.1.1. Preparation of ash gourd extracts

Ash gourd (5 kg) was cut in small pieces and soaked in aqueous methanol (1:4 v/v) overnight. The supernatant was removed by filtration through a sintered funnel under vacuum and the remaining ash gourd was crushed in an omni mixer for 2 min at a speed corresponding to position of the knob at 3. The slurry was filtered as above and the residue was further re-extracted twice with aqueous methanol. The filtrates were pooled and concentrated under vacuum on a rotary evaporator (35 °C) to obtain an aqueous solution.

The aqueous solution so obtained (500 ml) was filtered and then extracted with n-hexane (3 x 250 ml) in a separating funnel. The organic layers were pooled, washed with water (2 x 250 ml) and then evaporated to dryness under vacuum in a flash evaporator. The residue was made to 1% solution in DMSO. The remaining aqueous solution was consecutively extracted with ethyl acetate (3 x 250 ml) and n-butanol (3x 250 ml) as above. The organic layers in each case as well as the remaining aqueous solution were separately evaporated to dryness as above and made to 1% solution in ethanol. These fractions were tested for their growth promoting activity.

2.2.3.1.2. Activity Guided Fractionation

2.2.3.1.2.1. Growth promotion activity: Growth promotion activity was examined using tobacco plant because of its extensive and well characterized genetic background. Tobacco leaves were cut into circular discs of diameter 0.5 cm with the help of a sterile metallic cork borer. Discs obtained from the middle regions (between midrib and edge) of tobacco leaves were treated by soaking them overnight in MS liquid medium containing different concentrations of all the above extracts (0.178 – 4.4 µg/ml). Leaf discs soaked overnight in liquid media were used as control. The flasks were kept overnight on an orbital shaker at 100 rpm and maintained at 25 °C. The treated leaf discs were transferred to petri plates having half strength MS media containing 0.2 % agar, 3 % sucrose and 0.04% calcium chloride, adjusted to pH 5.7. The plates were then sealed with parafilm and kept in growth chambers under a 16 h photoperiod at 25 ± 1 °C for 21 d. The diameter of the incubated leaf discs were measured on day 3, 7, 14 and 21. Except for the total aqueous extract and the butanol extract, no increase in leaf disc diameter was observed with other fractions. The total aqueous extract as well as the butanol extract exhibited similar activity with an increase in diameter of tobacco leaf discs up to 1.1 cm on day 14. The leaves were found to undergo senescence beyond the period of 14 days.

2.2.3.1.2.2. TLC analysis

The butanol fraction was subjected to thin layer chromatography. Analytical TLC was carried out on ammonium sulphate impregnated silica gel G plate (10 cm x 25 cm x 0.25 mm thickness) using toluene:ethanol:formic acid (60:35:5, v/v/v) as the developing

solvent system. The separated spots were visualized by heating the plate in an oven at 120 °C for 20 min. The butanol extract was subsequently purified by preparative thin layer chromatography. Preparative TLC was carried out on silica gel plates (20 cm x 20 cm x 0.5 mm thickness) using the same solvent system as above. Five distinctly resolved bands were scrapped and eluted with methanol. The eluate was evaporated to dryness to make 1% solution (w/v).

2.2.3.1.2.3. Estimation by densitometry

Density of the individual spots/ bands was determined on a dual wavelength flying spot scanning densitometer Shimadzu CS-9301PC (Shimadzu, Kyoto, Japan). The density of the spots was determined in the reflectance mode at a wavelength of 528 nm. Acetoin glucoside was used as external standard. Aliquots of suitably diluted sample were spotted on the plate in increasing concentration ranging from 0.05 to 6 mg/ml. The amount of different bands was estimated from a standard curve (R^2 = 0.99) of spot density versus concentration and expressed as mg kg^{-1} of ash gourd.

2.2.3.1.2.4. Determination of active principles

The bands at Rf values 0.38, 0.44, 0.52, 0.59 and 0.77 were scrapped from the plate and eluted separately with distilled ethanol. Each solution was filtered, concentrated and made to 1% solution in ethanol. These fractions were tested for their growth promoting activity in order to identify the most active fraction. The growth promoting activity mainly resided in the band at Rf 0.44 which showed an increase in diameter of leaf discs up to 1.13 cm as compared to control (0.58 cm) on day 14. However, no increase in leaf disc diameter was observed when treated with the other four bands. Identification of the

active compound present in the TLC band at Rf 0.44 was further carried out as described below.

2.2.3.1.3. Structure identification

The preliminary identification of the compound present in the TLC band at Rf 0.44 was performed by GC/MS after acidic hydrolysis with the procedure as discussed earlier (section 2.10.2.3). The compound was tentatively identified as acetoin glucoside. The remaining aqueous solution from the individual bands was neutralized with 1 N KOH, dried under vacuum and the residue was dissolved in methanol. This methanol solution was subjected to TLC in order to identify the sugar residue. The sugar residue obtained from the hydrolyzate from the individual bands was further converted to its acetyl derivative using pyridine : acetic anhydride (1:1) (overnight, room temperature). The acetylated derivative was concentrated by slow stream of nitrogen and then subsequently analyzed by GC/MS.

Identity of the active compound present in this band was substantiated by other spectral techniques such as IR, NMR and further by chemical synthesis.

2.2.3.1.3.1. Spectral Studies

IR spectrum was recorded with a JASCO FTIR 4100 spectrophotometer (Jasco Corporation, Tokyo, Japan). νmax (KBr): 3520, 2980, 1665, 1452, 1385, 1180, 1114 and 910 cm^{-1}.

The NMR spectra were recorded with a Bruker AC-200 MHz FT NMR spectrometer (Bruker, Fallanden, Switzerland). The usual abbreviations employed are: s = singlet, d = doublet, q = quartet, J = coupling constant (in Hz), δ = chemical shift in ppm. The

proton designations as per chemical structure depicted in Fig. 10. ^{1}H NMR ($CDCl_3$, 200 MHz): δ ppm, 4.5-5.2 (sugar protons, H2', H3', H4', H5' & H6'), 4.22 (1H, q, H3, J = 5.4), 4.2 (1H, d, J = 5.8, H1'), 2.01 (3H, s, H1), 1.30 (3H, d, J = 5.4, H4).

2.2.3.1.3.2. Chemical synthesis of acetoin glucoside

Further confirmation of the structure was carried out by chemical synthesis according to the scheme depicted in Figure 11. In a 3- necked round bottom reaction flask, continuously flushed with argon, a mixture of sodium hydride in dry THF was added. Acetoin (88 mg) was then introduced into the flask in a controlled manner by using pressure equalizer. The reaction mixture was refluxed for 1h until the added acetoin was converted to its corresponding sodium derivative and effervescence of hydrogen had stopped. Acetobromo-D-glucose (397 mg) in THF was then added drop wise with the help of pressure equalizer with continuous stirring at room temperature for the formation of glycosidic bond between glucose and sodium derivative of acetoin. The reaction product was purified by column chromatography using binary solvent combination (15% ethyl acetate in petroleum ether) followed by preparative TLC using ethyl acetate: petroleum ether (15:85, v/v) as developing solvent. The final purified product was then characterized by spectral analysis as above.

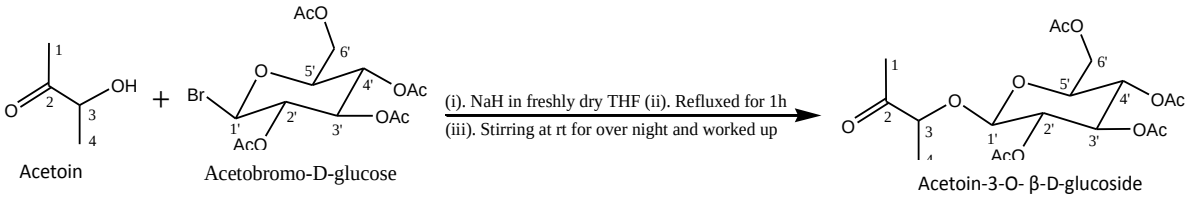


Figure 11. Scheme of chemical synthesis of acetoin-3-O-β-D- glucoside.

2.2.3.1.4. Growth promotion assay of purified acetoin glucoside

Tobacco leaf discs were treated with synthetic acetoin glucoside and that isolated from ash gourd, as well as standard acetoin procured from Sigma Aldrich, at concentrations ranging from 0.178 – 4.4 µg/ml. Leaf discs were cultured in similar growth conditions as mentioned above. The growth of the leaf discs was monitored at different time intervals (3, 7, 14 and 21 days). The growth observed was identical in both natural and synthetic acetoin glucoside. Therefore glucoside obtained from the natural source was used in subsequent studies. Three independent sets of experiments were performed and the data presented as a mean ± standard deviation.

2.2.3.1.5. Protein extraction and two-dimensional gel electrophoresis

Proteins were extracted using 0.1 M phosphate buffer supplemented with 0.1% Triton-X 100, 20 mM EDTA, 0.1 M NaCl, 5 mg/ml sodium ascorbate and 2 mM PMSF. Control and treated in vitro grown plant tissues (1 g fresh weight) were ground with liquid nitrogen using sterilized mortar and pestle. The powder was suspended in 1 ml of the above buffer. After incubating for 2 h at 4˚C, the samples were centrifuged at 12,000 g at 4˚C for 30 min. The centrifugation was repeated with the supernatant. The final supernatant was collected and equal amount of protein (90 µg) was used for iso-electric focusing (IEF). 2-D gel electrophoresis and IEF was done using an 11 cm strip as reported earlier [140] with the following focusing conditions- 250 V, 15 min (rapid, salt removal step); 8000 V, 2h (linear); 8000 V, 20-30,000V-h (rapid) and finally 500 V, hold. 2-D separation was done on a 12% PAGE gel and was silver stained.

2.2.3.1.6. Mass Spectrometric analysis and database searching

Spots were excised manually from gels and digested with trypsin. The digestion protocol used was as per the procedure reported earlier [141] with minor modifications. Gel plugs were initially destained with 20 mM ammonium bicarbonate. Trypsin (250 ng), at a concentration 25 ng/µl, was then added and the digestion proceeded at 37°C for 16 hrs. Peptides were extracted from gel plugs by adding 0.1% TFA containing 50% ACN/Water. Peptide fragments from digested proteins were then crystallized with saturated α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution. The MS analysis was performed in 4800 MALDI-TOF/TOF (Applied Biosystems, Foster City; CA) mass spectrometer in the m/z range 600-4500 Da. Peptide mass fingerprinting (PMF) search was performed using Swissprot database, using the MASCOT search engine (Matrix Science Ltd., London; http:/www.matrixscience.com).

2.2.3.1.7. Statistical Analysis

Experimental data of growth promotion studies were analyzed statistically by two-way ANOVA using DSAASTAT (Version 1.1) [142] to determine the effect of various treatments at different concentrations during the observed growth period. Multiple means comparison was done by Duncan's multiple range test ($p \leq 0.05$).

2.2.3.2. Compounds with ACE inhibition activity

2.2.3.2.1. Preparation of ash gourd extracts

The preparation of ash gourd extract and its subsequent partitioning with n-hexane, ethyl acetate and n-butanol was performed as explained in section 2.2.3.1.1. The organic

layers in each case as well as the remaining aqueous solution were separately evaporated to dryness and made to an aqueous solution (100 mg/ml). These fractions were tested for ACE inhibition activity.

2.2.3.2.2. Preparation of enzyme extract

Angiotensin converting enzyme (ACE) was extracted from pig *(Sus scrofa)* lung tissue. 10 g pig *(Sus scrofa)* lung tissue was minced using scissors on an ice bath and made to a volume of 100 mL with 10 mM sodium phosphate buffer (pH 8.3). The mixture was homogenized in chilled conditions. The homogenate was centrifuged at 21000 x g, for 20 min at 4 °C. The supernatant obtained was used as enzyme extract and stored at -20 °C till the analysis.

2.2.3.2.3. ACE inhibition assay

The ACE inhibition activity was measured using the procedure reported by Cushman and Cheung [143] with slight modifications. 50 µL of enzyme preparation was mixed with 50 µL of inhibitor (ash gourd extract) and pre-incubated at room temperature for 10 min. The mixture was designated as "test". A mixture of 50 µL distilled water + 50 µL enzyme was taken as control. 250 µL of N-Hippuryl-His-Leu tetrahydrate (HHL) as substrate (prepared in HEPES buffer) was added to the pre-incubated test and control mixture and incubated at 37 °C for 1 h. After 1 h, the reaction was stopped by adding 200 µL of 1 N HCL in each tube. 1 mL of ethyl acetate was then added and mixed thoroughly by vortexing for 90 s. They were then centrifuged at 21000 x g for 10 min. 700 µL aliquot from supernatant of each tube was collected in separate tubes and evaporated to dryness in a boiling water bath. After complete drying, 1 mL distilled

water was added in each tube and OD of aqueous solutions so obtained was read at 228 nm using UV spectrophotometer (Helios α). The assay was performed in triplicate for both sample and control tubes. The % inhibition activity was calculated as: [(OD_C - OD_T)/ OD_C]*100. The ACE inhibition activity mainly resided in the aqueous extract obtained after hexane, ethyl acetate and butanol fractionation. This aqueous extract was therefore further fractionated for determining the active principles.

2.2.3.2.4. Activity guided fractionation and identification of active principles

HPLC and GPC: The aqueous extract after fractionation with organic solvents as above was injected in RP-HPLC equipped with a hypercarb C18 column (150 mm x 4.6 mm length, 5 µm particle). The solvent system employed for elution consisted of a gradient of 0.1 % TFA and acetonitrile at a flow rate of 1 mL/min. The peaks were monitored at 220 nm. The major peaks observed were collected in separate tubes and lyophilized. The dry residue was then reconstituted in appropriate volume of distilled water and checked for ACE inhibition activity. The activity mainly resided in a peak at retention time 2.9 min. This peak was further fractionated using gel permeation chromatography with a Superdex^{TM} peptide 10/300 GL column. Out of several collected peaks from the column, the activity was present mainly in a peak at retention time of 30 min. This peak was repeatedly purified using GPC and subjected to TLC and GC/MS analysis.

TLC analysis: The aqueous extract obtained after partitioning with organic solvents and the purified peak obtained from GPC as above was spotted on an analytical TLC plate using toluene: ethanol: formic acid (6: 3.5: 0.5) as the developing solvent. After

development, the plate was dried at room temperature and then sprayed with 0.5 % ninhydrin solution in acetone for visualization.

GC/MS: The purified peak was subjected to mass spectroscopy by using direct probe facility available with the GC/MS (Schimadzu GC-17A). The active principles were thus tentatively identified by analyzing the mass spectral data obtained.

(B) Pumpkin

2.2.3.3. Isolation and identification of phenolic constituents

The methodologies adopted for isolation and identification of major phenolic compounds in pumpkin were similar to that as described in section 2.2.3.1.

2.2.3.4. Isolation and identification of carotenoids

2.2.3.4.1. Isolation of carotenoids

Ten grams of pumpkin from each tray was extracted twice with 10 mL hexane containing 10 mg of butylated hydroxytoluene (BHT). The extract was then centrifuged (16000 x g, 20 min, 4 °C) and the supernatant was collected. It was then passed through the anhydrous sodium sulphate. The extract so obtained was evaporated to dryness under vacuum (T < 30 °C) and the residue was made to 0.05% in a solvent mixture consisting of acetonitrile: tetrahydro furan (THF): methanol: 1% aq. ammonium acetate solution: BHT (55:35:5:5:0.05, v/v/v/v/w) as reported by Seo et al. [144]. Finally the solutions were filtered through 0.22 µm membrane filters (Millipore) prior to injection (20 µl) into the HPLC.

2.2.3.4.2. HPLC analysis

Carotenoids were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Jasco, Tokyo, Japan), equipped with a degasser, a quaternary pump delivery system, and a diode array detector. Separations were conducted using C-18 column (250 mm × 4.6 mm length, 5 μm particle size) with a solvent flow rate of 1 ml/min. The solvent system used for carotenoid analysis consisted of solvent A- acetonitrile: THF: methanol: 1% aqueous ammonium acetate solution: BHT (85:5:5:5:0.05, v/v/v/v/w) and solvent B- acetonitrile: THF: methanol: 1% aq ammonium acetate solution: BHT (55:35:5:5:0.05, v/v/v/v/w) [144]. The gradient employed was as follows: 0-10 min, 5% B; 10-29 min increasing linearly to 95% B; 29-35.9 min, maintaining 95% B; decreasing to 60 % B at 36 min; maintaining 60% B from 36-44.9 min. Carotenoids were monitored at 452 nm. The peaks were identified by comparing retention times and UV-Vis spectra in the 200-500 nm range with those of the corresponding authentic standards. Quantification of identified compounds was performed using calibration curves of the corresponding standard compounds at the specific absorption maximum. Co-chromatography with added authentic standards was also performed for further confirmation of the identified carotenoids.

2.2.3.5. Isolation and identification of triterpenes

2.2.3.5.1. Isolation of triterpenes

Pumpkin sample (10 g) from each tray was extracted twice with 20 mL methanol using a high speed omni mixer. The homogenate obtained was filtered through double layered washed and dried muslin cloth. The extract obtained was centrifuged (12,000 rpm, 20

°C, 15 min) and solution thus obtained was evaporated to dryness under vacuum (40 mbar, 40 °C) using a rotary evaporator (Buchi, Switzerland). The dried residue was made to 1% solution in methanol.

2.2.3.5.2. Identification of bioactive triterpenes

TLC analysis: The methanol extracts obtained as above were spotted on an analytical TLC (silica G, 0.25 mm) plate using toluene: ethanol: formic acid (5:4:1) as the developing solvent system. The developed plates were dried at room temperature and stained with iodine vapors. The spots so obtained were scanned on a Flying Spot Scanning Densitometer (CS9301PC, Shimadzu, Japan) and the area of each spot was then estimated. After the iodine vapors faded away, the plate was sprayed with Liebermann-Burchard reagent (5 mL acetic anhydride + 5 mL conc. H_2SO_4 + 50 mL ethanol), and visualized by heating the plate in oven at 120 °C for 20 min. The methanol extract was concentrated to dryness using rotary evaporator and hydrolyzed by pectinase enzyme (section 2.2.2.2.2.3.) The hydrolyzate along with the original methanol extract was again subjected to analytical TLC with different solvent systems (1- ethyl acetate : benzene (75:25) ; 2- chloroform : acetone (90:10)) to determine the nature of the compounds. Tentative identification of the resolved bands was performed by matching their R_f values available in literature.

HPLC analysis: The methanol extract as obtained above was subjected to reverse-phase high-performance liquid chromatography (RP-HPLC, Jasco, Tokyo, Japan), equipped with a degasser, a quaternary pump delivery system, and a diode array detector. Separations were conducted using C-18 column (250 mm × 4.6 mm length, 5 μm

particle size) with a solvent flow rate of 1 ml/min. An isocratic solvent system consisting of methanol: water (2:1) was used for elution. The peaks were monitored at 237 and 254 nm by a photo diode array detector operating in a wavelength range of 220-400 nm. Tentative identification of the peaks was performed on the basis of their retention times available in the literature.

GC/MS analysis: In order to further ascertain the identities of the active principles, the hydrolyzate of the total extract, as obtained above was analyzed by GC/MS. Identification was carried out by analyzing the mass spectral data thus obtained.

(C) Drumstick

2.2.3.6. Isolation, identification and quantification of phenolic constituents

The methodologies adopted for isolation and identification of major phenolic compounds in drumstick were similar to that as described in section 2.2.3.1.

2.2.3.7. Isolation and identification of glucosinolates

50 g of cut drumstick from each tray was taken in 85 ml of boiling water and subjected to microwave treatment for 3 min to inactivate the inherent myrosinase enzyme present in the vegetable. The mixture was then homogenized using a high speed homogenizer (B400, Buchi, Switzerland) and the slurry was filtered through double layered washed & dried muslin cloth. The extract obtained was centrifuged at 21000 x g at 20 °C for 15 min and subjected to HPLC and GC/MS analysis as briefly described below.

HPLC analysis: The aqueous glucosinolate extract was subjected to HPLC (Jasco HPLC system, Japan). Samples were eluted from a reverse phase C18 column (250 mm x 4.6 mm, 10 µ; HYPERSIL, Chromato-pack, Mumbai, India) using 0.1 % TFA in water as solvent A and 0.1 % TFA in methanol as solvent B. The solvent gradient employed was: At t = 0 - 5 min, A= 100%; t = 15-17 min, A = 83%; t=22 min, A = 75 %; t=30 min, A= 65%; t=35 min, A=50%, t=50 min, A=1%; t=55-60 min, A=100%, at a flow rate of 1.0 mL/min. Wavelength was set at 227 nm.

Glucosinolates were desulfated using 10 mL crude aqueous extract (10% solution) to which 500 µl of 0.02 M sulfatase enzyme in aq. NaOAc-AcOH (pH 5) was added and incubated overnight [145]. The resultant mixture was subjected to HPLC analysis as above for further confirmation of glucosinolates.

GC/MS analysis: 15 ml of the crude aq extract was evaporated to dryness in rotavapor and then reconstituted in 15 mL of 0.1 M phosphate buffer (pH 7.0). It was then added with 2 mg of myrosinase (Sigma) and incubated at 37 °C for 3 h. After completion of hydrolysis, samples were equilibrated for 45 min at 30 °C with continuous shaking and headspace was subsequently extracted by preconditioned PDMS/DVB/CAR fibres (Supleco, USA) for 20 min at 30 °C. After extraction, fibres were desorbed (270 °C) in injection port of GC/MS. 2-octanol (1.25 µg) was used as internal standard for quantification. Peak areas of compounds were estimated by comparing with those of internal standard and results reported as µg kg^{-1}.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Development of radiation processed shelf stable RTC vegetables

Five popular Indian vegetables - ash gourd (*Benincasa hispida*), pumpkin (*Cucurbita pepo*), bottle gourd (*Lagenaria siceraria*), lady finger (*Abelmoschus esculentus*) and drumstick (*Moringa oleifera*) were selected for the preliminary studies. Besides their nutritive value, these vegetables have also been used traditionally for their pharmacological and medicinal value. All the five vegetables were processed as described in materials and methods (section 2.2.1.) and stored at 10 °C. The samples were monitored at suitable intervals visually as well as by the sensory panel for appearance, color, texture and aroma. In the case of ready-to-cook (RTC) ash gourd, pumpkin and drumstick, radiation processed samples were acceptable up to a storage duration of 10-25 d depending on the vegetable and radiation dose delivered. In case of bottle gourd, the cut vegetable (control as well as radiation processed) developed browning / blackening after a few minutes of cutting, making them unacceptable. In case of ladies finger, the treated and untreated samples were acceptable only up to a storage period of 1-2 d, beyond which increased sliminess and off odor in the radiation treated samples restricting their acceptance by the sensory panel. Preliminary investigations thus showed that radiation processing was not amenable for the shelf life extension of RTC bottle gourd and lady finger. Hence ash gourd, pumpkin and drumstick were selected for the further studies.

3.1.1. Optimization of radiation dose and storage period with mathematical modeling

The shelf-life of fresh-cut fruits and vegetables depends on various factors such as processing conditions, treatment, storage temperature, packaging conditions, microbial

and physiological spoilage, that affect visual appearance, firmness and consumer acceptance. Understanding the response of a food system to qualitative or quantitative experimental variables is a complex task. Efficiency of mathematical modeling to optimize the processing conditions based on various experimental designs has been widely demonstrated [146]. Such mathematical equations can be utilized in the food industry for deciding the process parameters required for obtaining the desired shelf-life of fresh-cut produce, thus reducing severity of treatments.

In the present case, full factorial experimental design approach was employed for optimization of radiation dose and storage time for obtaining RTC products of the selected vegetables with acceptable microbial and sensory quality. A total of 90 experiments were performed for each vegetable at six radiation doses (0 – 2.5 kGy, at an interval of 0.5 kGy) and five storage days (0, 5, 8, 12 and 15 for ash gourd and drumstick; 0, 7, 14, 21 and 28 for pumpkin). The samples at each combination of dose and storage day were analyzed in triplicates for microbial quality (Total plate count (TPC) and Yeast & mold count (Y&MC)), color, texture and sensory acceptability as per procedure explained in materials and methods (section 2.2.1.). The experimental design and data obtained for ash gourd, drumstick and pumpkin is presented in Tables 17, 18 and 19, respectively.

The data obtained for each quality parameter (TPC, Y&MC, color, texture and overall acceptability) was individually fitted in cubic polynomial equations and models were generated. These polynomial equations were used to explain the complex interactions among radiation dose, storage duration and quality attributes of the RTC products.

Statistical parameters of the models generated for ash gourd, drumstick and pumpkin are represented in Tables 20, 21 and 22, respectively. Statistical analysis by ANOVA showed that the mathematical models generated for each product were significant ($p < 0.05$), while lack of fit was insignificant ($p > 0.05$). Further, the signal to noise ratio (S/N) for all models were above 4 indicating sufficient data to navigate designed space. Coefficients for predicted regression models and coefficient of determination (R^2) are shown in Tables 23, 24 and 25 for ash gourd, drumstick and pumpkin respectively. Good fitting of models is also indicated by high values of R^2.

Table 17. Experimental design for RTC ash gourd

Std	Run	Factor 1 A:Dose (kGy)	Factor 2 B: Storage duration (d)	TPC (Log CFU/g)	Y & M C (Log CFU/g)	L value	Firmness (N)	Overall acceptability
1	1	0(-1)	0(-1)	3.87	2.09	68.41	3.475	7.7
2	2	0(-1)	0(-1)	3.79	2.16	68.31	3.584	7.6
3	3	0(-1)	0(-1)	3.92	2.05	69.65	3.506	7.8
4	4	0.5(-0.6)	0(-1)	3.4	1.37	68.43	3.096	7.5
5	5	0.5(-0.6)	0(-1)	3.27	1.25	67.88	2.994	7.7
6	6	0.5(-0.6)	0(-1)	3.22	1.22	67.54	2.905	7.3
7	7	1(-0.2)	0(-1)	2.62	1	66.6	3.094	7.5
8	8	1(-0.2)	0(-1)	2.65	1	65.21	2.996	7.6
9	9	1(-0.2)	0(-1)	2.54	1	64.96	3.154	7.4
10	10	1.5(0.2)	0(-1)	2.63	1	68.54	2.844	7.3
11	11	1.5(0.2)	0(-1)	2.54	1	66.58	2.965	7.5
12	12	1.5(0.2)	0(-1)	2.54	1	67.45	2.881	7.1
13	13	2(0.6)	0(-1)	2	0.65	65.99	2.359	6.8
14	14	2(0.6)	0(-1)	1.85	0.7	64.69	2.296	7.1
15	15	2(0.6)	0(-1)	1.75	0.8	66.21	2.408	6.5
16	16	2.5(1)	0(-1)	1.06	0.5	66.65	2.5	5.2
17	17	2.5(1)	0(-1)	0.96	0.5	63.32	2.25	5.6
18	18	2.5(1)	0(-1)	1.11	0.5	67.12	2.21	4.8
19	19	0(-1)	5(-0.33)	8.71	2.07	63.52	2.475	1.9
20	20	0(-1)	5(-0.33)	8.54	1.95	62.54	2.562	2.1
21	21	0(-1)	5(-0.33)	8.13	2.06	61.38	2.333	1.7
22	22	0.5(-0.6)	5(-0.33)	6.38	2.04	64.71	2.839	3.1
23	23	0.5(-0.6)	5(-0.33)	6.58	2.15	64.17	2.882	3.3
24	24	0.5(-0.6)	5(-0.33)	5.85	1.95	63.22	2.915	2.9
25	25	1(-0.2)	5(-0.33)	5.97	2.15	67.96	2.889	5.4
26	26	1(-0.2)	5(-0.33)	5.56	1.96	67.55	2.914	5.6
27	27	1(-0.2)	5(-0.33)	5.03	2.2	66.95	2.814	5.2
28	28	1.5(0.2)	5(-0.33)	4.05	1.85	67.2	2.787	4.3
29	29	1.5(0.2)	5(-0.33)	3.95	1.75	67.55	2.669	4.4
30	30	1.5(0.2)	5(-0.33)	4.15	1.88	66.19	2.751	4.2
31	31	2(0.6)	5(-0.33)	2.16	1	61.73	2.354	7.1
32	32	2(0.6)	5(-0.33)	2.05	1	62.52	2.145	7.2
33	33	2(0.6)	5(-0.33)	2.2	1	63.51	2.262	7
34	34	2.5(1)	5(-0.33)	2.38	1	62.82	2.063	5.6
35	35	2.5(1)	5(-0.33)	2.35	1	63.98	1.996	6.2
36	36	2.5(1)	5(-0.33)	2.3	1	64.21	2.145	5
37	37	0(-1)	8(0.067)	9.62	3.78	52.32	1.075	2
38	38	0(-1)	8(0.067)	9.52	3.75	50.14	1.339	2.4
39	39	0(-1)	8(0.067)	9.45	3.69	51.22	1.041	1.6
40	40	0.5(-0.6)	8(0.067)	7.6	3.16	65.22	1.543	1.5
41	41	0.5(-0.6)	8(0.067)	7.54	2.95	63.85	1.496	1.7
42	42	0.5(-0.6)	8(0.067)	7.25	2.96	62.15	1.518	1.3
43	43	1(-0.2)	8(0.067)	6.81	2.75	64.86	1.261	2
44	44	1(-0.2)	8(0.067)	6.52	2.59	62.33	1.295	2.3

45	45	1(-0.2)	8(0.067)	6.1	2.65	60.54	1.195	1.7
46	46	1.5(0.2)	8(0.067)	5.01	2.38	64.6	2.139	3.5
47	47	1.5(0.2)	8(0.067)	4.65	2.34	63.29	1.965	3.8
48	48	1.5(0.2)	8(0.067)	4.85	2.15	62.66	2.354	3.2
49	49	2(0.6)	8(0.067)	3.62	1	58.13	2.565	7.1
50	50	2(0.6)	8(0.067)	3.56	1	55.82	2.592	7.2
51	51	2(0.6)	8(0.067)	3.45	1	57.46	2.663	7
52	52	2.5(1)	8(0.067)	3.01	1	54.86	2.507	5.5
53	53	2.5(1)	8(0.067)	2.95	1	52.61	2.658	5
54	54	2.5(1)	8(0.067)	2.78	1	55.83	2.214	6
55	55	0(-1)	12(0.6)	11.48	5.17	48.65	0.475	1.2
56	56	0(-1)	12(0.6)	11.25	5.05	45.18	0.337	1.3
57	57	0(-1)	12(0.6)	10.96	4.95	41.21	0.321	1.1
58	58	0.5(-0.6)	12(0.6)	8.55	4.41	51.99	0.909	1.5
59	59	0.5(-0.6)	12(0.6)	8.21	4.35	52.09	0.958	1.9
60	60	0.5(-0.6)	12(0.6)	8.65	4.25	50.64	0.861	1.1
61	61	1(-0.2)	12(0.6)	8.03	2.5	55.72	1.355	1.2
62	62	1(-0.2)	12(0.6)	7.95	2.35	54.68	1.339	1.3
63	63	1(-0.2)	12(0.6)	7.85	2.25	52.16	1.254	1.1
64	64	1.5(0.2)	12(0.6)	6.13	2.32	63.47	1.936	1.4
65	65	1.5(0.2)	12(0.6)	5.96	2.25	61.95	1.99	1.6
66	66	1.5(0.2)	12(0.6)	6.11	2.06	59.76	1.854	1.2
67	67	2(0.6)	12(0.6)	3.98	2.34	62.75	2.066	6.8
68	68	2(0.6)	12(0.6)	3.58	2.15	60.22	2.054	7
69	69	2(0.6)	12(0.6)	3.75	2.5	59.17	1.965	6
70	70	2.5(1)	12(0.6)	3.25	2.02	58.73	1.549	4.2
71	71	2.5(1)	12(0.6)	3.05	1.99	55.43	1.543	4.4
72	72	2.5(1)	12(0.6)	2.99	1.85	56.33	1.354	4.5
73	73	0(-1)	15(1)	12.52	6.12	42.65	0.325	1
74	74	0(-1)	15(1)	11.96	6.06	39.18	0.187	1
75	75	0(-1)	15(1)	11.24	5.98	35.21	0.171	1
76	76	0.5(-0.6)	15(1)	9.68	4.99	45.99	0.759	1
77	77	0.5(-0.6)	15(1)	9.25	5.21	46.09	0.808	1
78	78	0.5(-0.6)	15(1)	8.96	5.11	44.64	0.711	1
79	79	1(-0.2)	15(1)	8.96	3.25	49.72	1.205	1
80	80	1(-0.2)	15(1)	9.02	3.16	48.68	1.189	1
81	81	1(-0.2)	15(1)	9.62	3.1	46.16	1.104	1
82	82	1.5(0.2)	15(1)	6.99	2.86	57.47	1.786	1
83	83	1.5(0.2)	15(1)	6.26	2.72	55.95	1.84	1
84	84	1.5(0.2)	15(1)	7.06	2.92	53.76	1.704	1
85	85	2(0.6)	15(1)	4.56	2.66	56.75	1.916	4
86	86	2(0.6)	15(1)	5	2.59	54.22	1.904	3
87	87	2(0.6)	15(1)	4.69	2.34	53.17	1.815	4
88	88	2.5(1)	15(1)	4.03	2.35	52.73	1.399	4
89	89	2.5(1)	15(1)	4.27	2.15	49.43	1.393	2
90	90	2.5(1)	15(1)	3.99	2.22	50.33	1.204	5

Table 18. Experimental design for RTC drumstick

Std	Run	Factor 1 A:Dose(kGy)	Factor 2 B: Storage period (d)	TPC (Log CFU/g)	YMC (Log CFU/g)	a value	Firmness (N)	Overall acceptability
1	1	0(-1)	0(-1)	5.613	2.864	-4.5	7.46	8
2	2	0(-1)	0(-1)	5.426	2.694	-4.5	7.23	7.5
3	3	0(-1)	0(-1)	5.501	2.774	-4.5	7.3	7.5
4	4	0.5(-0.6)	0(-1)	2.954	1.589	-4.5	6.88	7
5	5	0.5(-0.6)	0(-1)	3.614	2.109	-4.5	6.81	7.5
6	6	0.5(-0.6)	0(-1)	2.931	1.842	-4.5	6.69	7
7	7	1(-0.2)	0(-1)	2.662	1.265	-4.5	7.38	7
8	8	1(-0.2)	0(-1)	2.601	1.291	-4.5	7.14	7.5
9	9	1(-0.2)	0(-1)	2.706	0.956	-4.5	7.22	7
10	10	1.5(0.2)	0(-1)	2.109	0.782	-4.5	6.62	6
11	11	1.5(0.2)	0(-1)	2.236	0.891	-4.5	6.35	6.5
12	12	1.5(0.2)	0(-1)	2.365	1	-4.5	6.42	6.5
13	13	2(0.6)	0(-1)	0.756	0.295	-4.5	6.32	6
14	14	2(0.6)	0(-1)	0.702	0.383	-4.5	6.25	5.9
15	15	2(0.6)	0(-1)	0.824	0.364	-4.5	6.14	6
16	16	2.5(1)	0(-1)	0.262	0.195	-4.5	5.88	5.5
17	17	2.5(1)	0(-1)	0.278	0.236	-4.5	5.87	5.5
18	18	2.5(1)	0(-1)	0.283	0.264	-4.5	5.88	5.9
19	19	0(-1)	5(-0.33)	6.281	2.953	-3	6.61	5
20	20	0(-1)	5(-0.33)	6.255	2.576	-3	6.84	4.5
21	21	0(-1)	5(-0.33)	6.093	2.994	-3	6.67	5

22	22	0.5(-0.6)	5(-0.33)	4.125	2.105	-3	6.54	5.5
23	23	0.5(-0.6)	5(-0.33)	4.198	1.963	-3	6.59	5
24	24	0.5(-0.6)	5(-0.33)	4.396	2.201	-3	6.76	5
25	25	1(-0.2)	5(-0.33)	3.836	2.054	-4.5	7.25	7
26	26	1(-0.2)	5(-0.33)	3.725	2.114	-4.5	6.92	6.5
27	27	1(-0.2)	5(-0.33)	3.694	1.462	-4.5	7.14	6.5
28	28	1.5(0.2)	5(-0.33)	3.274	1.102	-4.5	6.57	5
29	29	1.5(0.2)	5(-0.33)	3.214	0.901	-4.5	6.4	5.5
30	30	1.5(0.2)	5(-0.33)	3.193	0.707	-4.5	6.37	5
31	31	2(0.6)	5(-0.33)	0.892	0.521	-4.5	5.81	4.5
32	32	2(0.6)	5(-0.33)	0.9	0.613	-4.5	6.06	5
33	33	2(0.6)	5(-0.33)	0.91	0.563	-4.5	5.86	4
34	34	2.5(1)	5(-0.33)	0.569	0.451	-4.5	4.79	4
35	35	2.5(1)	5(-0.33)	0.6321	0.396	-4.5	4.77	4
36	36	2.5(1)	5(-0.33)	0.612	0.391	-4.5	4.81	4
37	37	0(-1)	8(0.067)	7.894	3.348	-2.5	5.52	3
38	38	0(-1)	8(0.067)	7.921	2.865	-2.5	5.32	3
39	39	0(-1)	8(0.067)	7.723	3.287	-2.5	5.41	3
40	40	0.5(-0.6)	8(0.067)	6.653	2.371	-2.5	6.64	4
41	41	0.5(-0.6)	8(0.067)	6.98	2.141	-2.5	6.34	4
42	42	0.5(-0.6)	8(0.067)	6.708	2.196	-2.5	6.59	4
43	43	1(-0.2)	8(0.067)	4.115	2.235	-4.5	7.06	6
44	44	1(-0.2)	8(0.067)	4.257	1.856	-4.5	6.87	6
45	45	1(-0.2)	8(0.067)	4.253	2.176	-4.5	7.09	6
46	46	1.5(0.2)	8(0.067)	3.627	1.784	-4.5	5.08	4
47	47	1.5(0.2)	8(0.067)	3.748	1.654	-4.5	4.77	4
48	48	1.5(0.2)	8(0.067)	3.269	1.765	-4.5	4.87	4
49	49	2(0.6)	8(0.067)	2.664	1.851	-4	3.59	3
50	50	2(0.6)	8(0.067)	2.851	1.844	-4	3.15	3
51	51	2(0.6)	8(0.067)	2.317	1.904	-4	3.17	3
52	52	2.5(1)	8(0.067)	1.564	2.589	-4	3.1	2
53	53	2.5(1)	8(0.067)	1.628	2.612	-4	3.11	2
54	54	2.5(1)	8(0.067)	1.596	2.575	-4	3.17	2
55	55	0(-1)	12(0.6)	8.902	3.512	-2	4.05	1
56	56	0(-1)	12(0.6)	8.602	3.201	-2	3.76	1
57	57	0(-1)	12(0.6)	9.214	2.896	-2	3.82	1
58	58	0.5(-0.6)	12(0.6)	8.458	2.965	-2	3.53	2
59	59	0.5(-0.6)	12(0.6)	8.217	2.331	-2	3.46	2
60	60	0.5(-0.6)	12(0.6)	8.394	2.813	-2	3.52	2
61	61	1(-0.2)	12(0.6)	4.189	2.514	-4	6.47	6
62	62	1(-0.2)	12(0.6)	4.324	2.019	-4	6.35	6.5
63	63	1(-0.2)	12(0.6)	5.014	2.369	-4	6.47	6
64	64	1.5(0.2)	12(0.6)	4.791	1.419	-4	3.17	3
65	65	1.5(0.2)	12(0.6)	4.932	1.126	-4	3.14	3
66	66	1.5(0.2)	12(0.6)	5.147	1.154	-4	3.1	3
67	67	2(0.6)	12(0.6)	2.982	2.204	-3.5	2.06	2
68	68	2(0.6)	12(0.6)	3.014	2.132	-3.5	2.03	2
69	69	2(0.6)	12(0.6)	3.141	2.195	-3.5	1.93	2
70	70	2.5(1)	12(0.6)	2.284	2.965	-3.5	1.86	2
71	71	2.5(1)	12(0.6)	2.096	2.925	-3.5	1.92	2
72	72	2.5(1)	12(0.6)	2.165	2.801	-3.5	1.87	2
73	73	0(-1)	15(1)	9.302	3.925	-1.5	1.77	1
74	74	0(-1)	15(1)	9.225	3.881	-1.5	1.81	1
75	75	0(-1)	15(1)	9.314	3.636	-1.5	1.85	1
76	76	0.5(-0.6)	15(1)	8.958	3.065	-1.5	2.87	1
77	77	0.5(-0.6)	15(1)	8.77	3.231	-1.5	2.74	1
78	78	0.5(-0.6)	15(1)	8.914	3.113	-1.5	2.77	1
79	79	1(-0.2)	15(1)	6.489	2.835	-3.5	6.32	1
80	80	1(-0.2)	15(1)	6.546	2.908	-3.5	6.26	1
81	81	1(-0.2)	15(1)	6.714	2.891	-3.5	6.21	1
82	82	1.5(0.2)	15(1)	5.791	2.619	-3	3.1	1
83	83	1.5(0.2)	15(1)	5.632	2.351	-3	2.94	1
84	84	1.5(0.2)	15(1)	5.947	2.524	-3	2.89	1
85	85	2(0.6)	15(1)	3.812	2.913	-3	1.79	1
86	86	2(0.6)	15(1)	3.951	2.826	-3	1.84	1
87	87	2(0.6)	15(1)	3.824	2.915	-3	1.91	1
88	88	2.5(1)	15(1)	3.834	3.25	-3.1	1.7	1
89	89	2.5(1)	15(1)	3.287	3.225	-3.1	1.67	1
90	90	2.5(1)	15(1)	3.294	3.281	-3.1	1.64	1

Table 19. Experimental design for RTC pumpkin

Std	Run	Factor 1 A:Dose (kGy)	Factor 2 B: Storage duration (d)	TPC (Log CFU/g)	Y & MC (Log CFU/g)	Lvalue	Firmness (N)	Overall acceptability
1	1	0(-1)	0(-1)	5.03	2.69	75.25	1.719	8
2	2	0(-1)	0(-1)	5.52	3.12	74.96	1.655	8.5
3	3	0(-1)	0(-1)	4.97	2.25	75.24	1.769	8.5
4	4	0.5(-0.6)	0(-1)	3.01	1.74	72.38	1.404	8.5
5	5	0.5(-0.6)	0(-1)	3.25	1.77	72.73	1.541	8
6	6	0.5(-0.6)	0(-1)	3.1	1.55	73.06	1.455	8
7	7	1(-0.2)	0(-1)	2.51	1.72	73.91	1.23	7.5
8	8	1(-0.2)	0(-1)	2.17	1.96	74.98	1.121	7
9	9	1(-0.2)	0(-1)	2.97	1.45	73.28	1.23	7.5
10	10	1.5(0.2)	0(-1)	2.21	1.41	75.25	0.907	6.5
11	11	1.5(0.2)	0(-1)	1.54	1.42	74.82	1.064	6.2
12	12	1.5(0.2)	0(-1)	1.82	1.28	73.86	1.025	6
13	13	2(0.6)	0(-1)	1.09	1.03	75.88	0.842	6.5
14	14	2(0.6)	0(-1)	1.52	1.01	75.03	0.768	6
15	15	2(0.6)	0(-1)	1.12	0.92	74.96	0.754	6
16	16	2.5(1)	0(-1)	0.64	0.39	75.26	0.678	5.2
17	17	2.5(1)	0(-1)	0.42	0.34	75.22	0.634	5.5
18	18	2.5(1)	0(-1)	0.63	0.52	74.021	0.724	5
19	19	0(-1)	5(-0.33)	5.35	4.05	79.23	1.766	7.5
20	20	0(-1)	5(-0.33)	4.95	4.59	78.33	1.806	7.2
21	21	0(-1)	5(-0.33)	5.59	3.56	77.8	1.769	7
22	22	0.5(-0.6)	5(-0.33)	3.25	2.62	78.66	1.216	6
23	23	0.5(-0.6)	5(-0.33)	3	2.25	79.61	0.995	6.2
24	24	0.5(-0.6)	5(-0.33)	3.32	3.26	78.98	1.332	6
25	25	1(-0.2)	5(-0.33)	2.78	2.68	80.52	1.125	7
26	26	1(-0.2)	5(-0.33)	2.25	2.35	80.72	1.245	7.5
27	27	1(-0.2)	5(-0.33)	2.99	2.85	81.42	1.235	7
28	28	1.5(0.2)	5(-0.33)	1.83	2.02	82.39	1.038	6.2
29	29	1.5(0.2)	5(-0.33)	2.13	1.88	75.35	0.9321	6
30	30	1.5(0.2)	5(-0.33)	2	0.94	77.93	0.802	6.2
31	31	2(0.6)	5(-0.33)	2.02	1.18	80.97	0.822	5
32	32	2(0.6)	5(-0.33)	1.63	0.96	70.82	0.967	5.2
33	33	2(0.6)	5(-0.33)	2.02	1.96	84.15	0.863	5
34	34	2.5(1)	5(-0.33)	0.93	0.86	72.33	0.654	5.5
35	35	2.5(1)	5(-0.33)	1.91	0.93	77.88	0.797	5
36	36	2.5(1)	5(-0.33)	1.13	1	79.38	0.779	5
37	37	0(-1)	8(0.067)	6.57	4.53	85.87	1.8104	1
38	38	0(-1)	8(0.067)	6.25	4.22	85.31	1.782	1.5
39	39	0(-1)	8(0.067)	7.01	4	86.13	1.955	1
40	40	0.5(-0.6)	8(0.067)	4.07	3.07	86.53	1.425	4
41	41	0.5(-0.6)	8(0.067)	4.56	3.32	84.77	1.04	3.5
42	42	0.5(-0.6)	8(0.067)	3.54	3.74	86.52	1.338	4
43	43	1(-0.2)	8(0.067)	3.63	2.76	84.66	1.128	7
44	44	1(-0.2)	8(0.067)	3.1	2.71	85.45	1.229	6.5
45	45	1(-0.2)	8(0.067)	3.66	2.77	84.57	1.312	7
46	46	1.5(0.2)	8(0.067)	3.06	2.49	81.4	0.729	5
47	47	1.5(0.2)	8(0.067)	2.93	2.82	78.691	1.034	5.2
48	48	1.5(0.2)	8(0.067)	2.68	2.23	89.653	0.934	5
49	49	2(0.6)	8(0.067)	2.37	3	85.965	0.836	4
50	50	2(0.6)	8(0.067)	2.13	1.21	85.319	0.921	3.5
51	51	2(0.6)	8(0.067)	1.96	1.93	76.342	0.768	4
52	52	2.5(1)	8(0.067)	2.15	1.51	74.923	0.759	4
53	53	2.5(1)	8(0.067)	2.01	1.43	79.642	0.734	3
54	54	2.5(1)	8(0.067)	1.83	1.52	85.819	0.603	4
55	55	0(-1)	12(0.6)	6.8	4.61	89.25	1.981	1
56	56	0(-1)	12(0.6)	7.31	4.22	88.39	1.89	1
57	57	0(-1)	12(0.6)	7.01	5.01	95.48	1.947	1
58	58	0.5(-0.6)	12(0.6)	6.51	3.16	89.54	1.245	3
59	59	0.5(-0.6)	12(0.6)	6.96	3.95	83.57	1.321	2.5
60	60	0.5(-0.6)	12(0.6)	6.21	2.96	84.21	1.129	3
61	61	1(-0.2)	12(0.6)	3.93	3.08	85.98	1.108	7
62	62	1(-0.2)	12(0.6)	4.13	3.37	86.21	1.02	6.5
63	63	1(-0.2)	12(0.6)	3.85	3	86.94	0.97	7
64	64	1.5(0.2)	12(0.6)	3.01	2.76	88.642	0.967	4
65	65	1.5(0.2)	12(0.6)	3.48	2.9	87.06	0.797	3
66	66	1.5(0.2)	12(0.6)	2.97	2.96	83.921	0.803	4

67	67	2(0.6)	12(0.6)	2.66	2.39	87.406	0.778	3
68	68	2(0.6)	12(0.6)	2.72	2.42	79.693	0.876	2.5
69	69	2(0.6)	12(0.6)	2.53	2.26	89.335	0.783	3
70	70	2.5(1)	12(0.6)	2.2	1.5	82.721	0.669	3
71	71	2.5(1)	12(0.6)	2.19	1.48	78.745	0.686	2.5
72	72	2.5(1)	12(0.6)	2.31	1.63	89.241	0.608	3
73	73	0(-1)	15(1)	9.85	6.4	96.21	2.1	1
74	74	0(-1)	15(1)	9.53	5.99	93.25	2.321	1
75	75	0(-1)	15(1)	9.07	6.21	90.28	2.206	1
76	76	0.5(-0.6)	15(1)	8.56	4.96	92.54	1.1235	1
77	77	0.5(-0.6)	15(1)	7.97	5.21	84.57	1.021	1
78	78	0.5(-0.6)	15(1)	7.87	5.17	85.21	0.987	1
79	79	1(-0.2)	15(1)	4.87	4.12	86.98	0.963	2
80	80	1(-0.2)	15(1)	5.96	3.99	87.21	0.859	2
81	81	1(-0.2)	15(1)	5.57	4.37	87.94	0.821	2
82	82	1.5(0.2)	15(1)	3.97	3.27	89.642	0.765	3
83	83	1.5(0.2)	15(1)	3.67	4.33	88.06	0.652	2.5
84	84	1.5(0.2)	15(1)	4.13	4.22	84.921	0.506	3
85	85	2(0.6)	15(1)	3.13	3.96	88.406	0.521	2
86	86	2(0.6)	15(1)	2.97	3.36	80.693	0.536	1.5
87	87	2(0.6)	15(1)	3.66	3.02	90.335	0.468	2
88	88	2.5(1)	15(1)	2.32	2.57	83.721	0.521	2
89	89	2.5(1)	15(1)	2.85	2.21	79.745	0.511	1
90	90	2.5(1)	15(1)	1.97	2.2	90.241	0.421	2

Table 20. Significance statistics, p values and signal to noise ratio (S/N) of predicted models for ash gourd

	Model			Lack of fit			S/N
Response	Sum of squares	df	p-value	Sum of squares	df	p-value	
TPC	742.38	7	0.0001	14.62	20	0.2501	65.713
Y&MC	155.62	9	0.0001	9.23	20	0.0621	49.464
Color (L)	4988.54	7	0.0001	440.88	22	0.07801	39.457
Firmness	55.79	8	0.0001	5.35	21	0.0001	40.374
Overall acceptability	469.62	9	0.0001	58.09	20	0.3243	23.791

Table 21. Significance statistics, p values and signal to noise ratio (S/N) of predicted models for drumstick

Model				Lack of fit			S/N
Response	Sum of squares	df	p-value	Sum of squares	df	p-value	
TPC	505.63	5	0.0001	13.89	24	0.3558	72.85
Y&MC	270.24	6	0.0001	18.18	23	0.0515	45.34
Color (a)	72.65	7	0.0001	12.66	22	0.0831	29.03
Firmness	524.69	6	0.0001	53.85	23	0.073	34.22
Overall acceptability	675.48	8	0.0001	32.99	21	0.0511	42.44

Table 22. Significance statistics, p values and signal to noise ratio (S/N) of predicted models for pumpkin

Model				Lack of fit			S/N
Response	Sum of squares	df	p-value	Sum of squares	df	p-value	
TPC	135.7176	8	0.0001	2.826047	15	0.1647	50.440
Y&MC	80.52517	6	0.0001	2.212739	17	0.4606	36.465
Color (L)	1652.848	3	0.001	97.26419	20	0.9714	22.343
Firmness	7.21269	6	0.0001	0.229633	17	0.1097	34.694
Overall acceptability	222.4349	7	0.0001	47.0651216	16		27.306

Table 23. Coefficients of the fitted polynomial representing the relationship between the response and the process variable and R^2 values for RTC ash gourd

Coefficients	**TPC (log cfu/g)**	**Y & MC (log cfu/g)**	**Color (L)**	**Firmness (N)**	**Overall acceptability**
Intercept	5.43[a]	1.97[a]	63.30[a]	2.21[a]	3.75[a]
A-Dose	-3.56[a]	-1.13[a]	1.12[a]	0.66[a]	4.09[a]
B-Storage time	2.08[a]	1.20[a]	-7.04[a]	-1.30[a]	-3.10[a]
AB	-1.29[a]	-0.67[a]	4.63[a]	0.71[a]	1.20[a]
A^2	0.38[a]	0.39[a]	-5.48[a]	-0.38[a]	0.055[a]
B^2	-0.51[a]	0.22[a]	-2.64[a]	0.00079	0.70[a]
A^2B	0.10[a]	0.58[a]	-4.15[a]	-0.35[a]	1.33[a]
AB^2	0.83[a]	-0.18[a]	1.91[a]	-0.35[a]	-2.34[a]
A^3	0.099 [a]	0.019[a]	0.01	-0.35[a]	-1.90[a]
B^3	0.43[a]	-0.19[a]	-0.01	0.64[a]	-0.53[a]
R^2	0.97	0.93	0.89	0.90	0.87

[a] Significant terms at $p \leq 0.05$

Table 24. Coefficients of the fitted polynomial representing the relationship between the response and the process variable and R^2 values for RTC drumstick

Coefficients	**TPC (log cfu/g)**	**Y & MC (log cfu/g)**	**Color (a)**	**Firmness (N)**	**Overall acceptability**
Intercept	-0.39[a]	-0.51[a]	-4.07[a]	1.46[a]	0.91[a]
A-Dose	-0.68[a]	-0.56[a]	-1.12[a]	-1.61[a]	-1.45[a]
B-Storage time	0.57[a]	0.57[a]	0.59[a]	-1.27[a]	-0.49

AB	0.018	1.15[a]	-0.38[a]	-0.056	2.97[a]
A^2	-0.100	0.62[a]	0.72[a]	-1.58[a]	-1.90[a]
B^2	0.12	0.019[a]	0.18[a]	-0.74[a]	1.49[a]
A^2B	2.49[a]	1.83[a]	0.45[a]	-2.96[a]	-2.19[a]
AB^2	-1.72[a]	-1.25[a]	0.48[a]	-0.96[a]	-2.13[a]
A^3	-1.24[a]	-0.34	0.27	0.64[a]	2.66[a]
B^3	0.22	0.26	0.14	0.073[a]	-1.24[a]
R^2	0.92	0.86	0.86	0.79	0.89

[a] Significant terms at $p \leq 0.05$

Table 25. Coefficients of the fitted polynomial representing the relationship between the response and the process variable and R^2 values for RTC pumpkin

Coefficients	**Logit(TPC)**	**Y&MC (log cfu/g)**	**Color (L)**	**Firmness (N)**	**Overall acceptability (Hedonic)**
Intercept	0.037187[a]	3.15638[a]	82.04992[a]	0.275679[a]	5.737103[a]
A-Dose	-0.87613[a]	-0.73809[a]	-0.80918[a]	-0.2271[a]	1.074074[a]
B-Storage time	0.984792[a]	0.857211[a]	8.792812[a]	-0.00235	-3.42333[a]
AB	-0.43095[a]	-0.04779	-0.8359[a]	-0.04877[a]	1.412679[a]
A^2	0.313907[a]	0.223595[a]	0.484688	0.051385[a]	-0.8006[a]
B^2	0.578462[a]	-0.43183[a]	-1.39156	0.018442	0.3375
A^2B	0.120244[a]	-0.00117	0.048437	0.014139[a]	-0.30804[a]
AB^2	-0.29278[a]	0.100796[a]	0.352516	-0.02633[a]	-0.225
A^3	-0.04786[a]	-0.04214[a]	-0.12251	-0.008[a]	0.113426[a]
B^3	-0.10923	-0.02724	-1.78247	-0.02758	1.3125[a]
R^2	0.937315	0.905814	0.730179	0.918311	0.825361

[a] Significant terms at $p \leq 0.05$

3.1.1.1. Microbial quality

Microbial contamination can be a major source of spoilage of fresh-cut produce. Microbial spoilage has been used by quality assurance departments in the fresh-cut industry as an objective indicator of quality failure of fresh-cut vegetable commodities [147]. Contamination sources of fresh-cut produce include raw materials and contact with processing equipment. The micro-organisms that exist on the surfaces of raw, whole produce appear to be the major source of microbial contamination and consequent spoilage of fresh-cut fruits and vegetables.

Fresh-cut vegetables have high levels of microorganisms and several outbreaks of food-borne illnesses have been found to be associated with these products (Fan et al., 2008 from intro). In general, total bacterial counts of fresh-cut products just after processing were reported to range from 3 to 6 log CFU g^{-1} [13]. There are no standard prescribed limits for acceptable microbial load in case of fresh fruits and vegetables. The upper limit for total microbial load of fresh-cut vegetables during the entire intended storage duration as set by European Standards is < 7 log CFU g^{-1} [148]. However, according to guidelines of the Centre for Food Safety, Hong Kong, the total microbial load of < 5 log CFU g^{-1} has been considered satisfactory for fresh-cut mixed salads [149]. Therefore, in the present study, a total microbial load of 5 log CFU g^{-1} was taken as the upper limit for microbial acceptability of RTC vegetables.

A significant ($p \leq 0.05$) increase in mesophilic bacterial (TPC) and yeast and mold counts (Y&MC) during storage was observed in non-irradiated control samples of all the three vegetables. Gamma irradiation significantly reduced microbial load and it decreased

linearly with radiation dose. The effect of radiation treatment and storage duration on TPC and Y&MC in ash gourd, drumstick and pumpkin is depicted in Figures 12, 13 and 14, respectively.

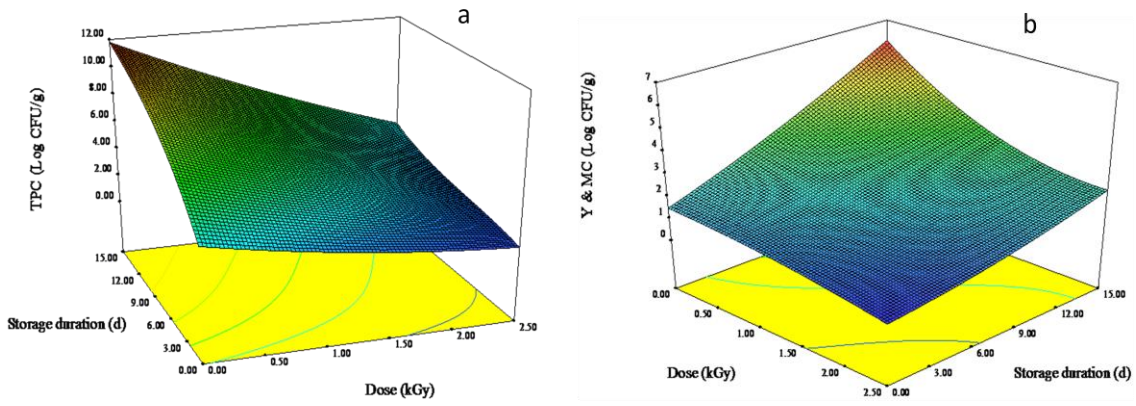


Figure 12. Variation of (a) Total plate counts (media: plate count agar, 37 °C, 24 h) and (b) yeast and mold counts (media: potato dextrose agar, 37 °C, 48 h) with radiation dose (0 – 2.5 kGy) and storage time (0 – 15 d) in RTC ash gourd.

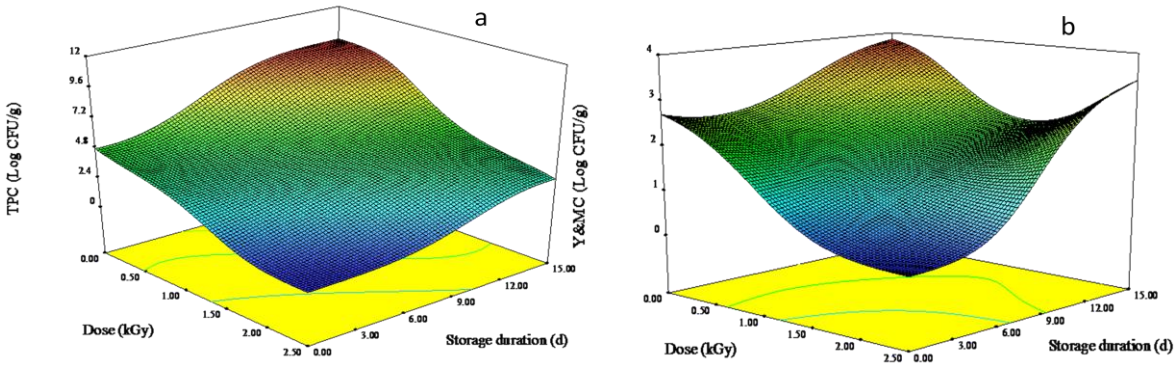


Figure 13. Variation of (a) Total plate counts (media: plate count agar, 37 °C, 24 h) and (b) yeast and mold counts (media: potato dextrose agar, 37 °C, 48 h) with radiation dose (0 – 2.5 kGy) and storage time (0 – 15 d) in RTC drumstick.

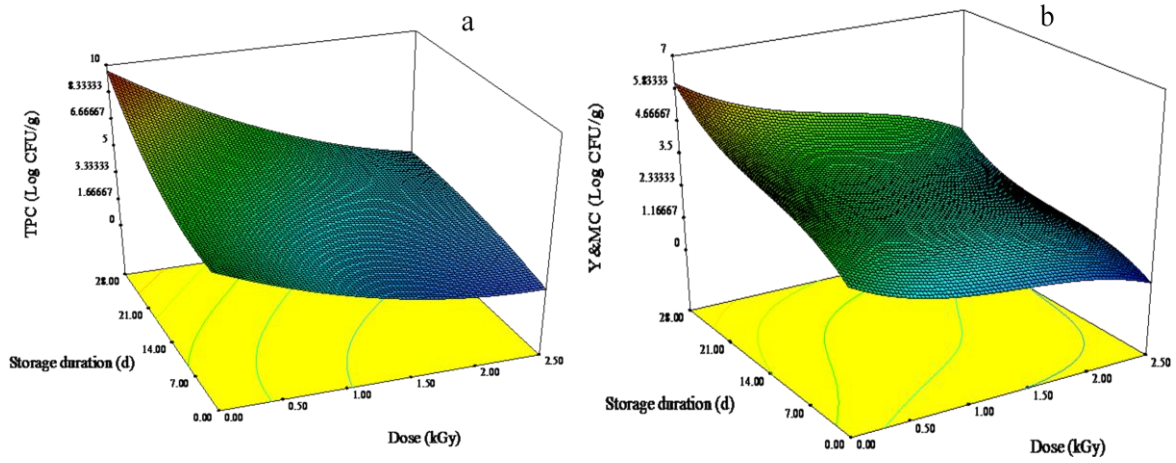


Figure 14. Variation of (a) Total plate counts (media: plate count agar, 37 °C, 24 h) and (b) yeast and mold counts (media: potato dextrose agar, 37 °C, 48 h) with radiation dose (0 – 2.5 kGy) and storage time (0 – 28 d) in RTC pumpkin.

Ash gourd: Control samples of RTC ash gourd on zero day of storage had TPC and Y&MC load of 3.86 and 2.09 log CFU/g, respectively. The mesophilic counts reduced by 2-3 log cycles compared to the control immediately after radiation treatment depending on the dose delivered (Figure 12 a). In control and samples irradiated at 0.5 and 1 kGy, counts reached higher than 7 log CFU/g, on day eight, beyond the acceptable limit prescribed (5 log CFU/g) for fresh cut vegetables [148, 149]. In samples exposed to a radiation dose of 1.5 kGy the mesophilic counts showed values greater than 5 log $CFUg^{-1}$ on day 12 of storage period, reducing the microbial quality of the product. Samples irradiated at 2 kGy or above exhibited no microbial spoilage until 12 d of storage. At the end of storage period (15 d), the counts exceeded the acceptable limit in control and samples irradiated below 2 kGy. However, a radiation dose of 2 kGy and above was suitable to keep the mesophillic counts well below the acceptable limit.

The response of yeast and mold counts for different radiation doses is represented in Figure 12 b. At 2 and 2.5 kGy, a reduction in counts by 2 log cycles was observed after irradiation on day zero. The low counts were maintained during storage in these samples as compared to the control and those irradiated at lower doses (0.5–1.5 kGy). Thus radiation treatment at 2 kGy and above was highly effective in reducing total mesophilic, and yeast & mold counts in RTC ash gourd.

Drumstick: The effect of radiation processing and storage duration on TPC and Y&MC load in RTC drumstick is depicted in Figure 13. On day zero, TPC and Y&MC load of control samples were 5.61 and 2.86 log CFU/g, respectively. Significant ($p < 0.05$) dose dependent reduction in mesophilic bacterial counts was observed in radiation processed samples. The mesophilic counts reduced by 3-5 log cycles immediately after radiation treatment compared to the control. A 3-log reduction was observed in mesophilic counts at a radiation dose of 1 kGy, while Y&MC decreased by 2 log cycles at this dose. During storage, a significant ($p < 0.05$) increase in TPC and Y&MC was observed in control as well as irradiated samples. However, radiation processed samples had a significantly lower counts as compared to control at all the storage days studied. At the end of storage period (15 d), the TPC exceeded the acceptable limit in control (> 9 log CFU/g) and samples irradiated at 0.5 kGy. However, a radiation dose of 1 kGy and above was suitable to keep the mesophillic counts well below the acceptable limit.

Pumpkin: The mesophilic bacterial and Y&MC counts were 5.12 and 2.7 log CFU/g respectively in control pumpkin on day zero. The effect of radiation treatment and storage duration on TPC and Y&MC is depicted in Figure 14. Radiation processing resulted in a

significant ($p < 0.05$) dose-dependent reduction in the microbial counts. A 4-log reduction was observed in mesophilic bacterial counts at a radiation dose of 2 kGy, while Y&MC decreased by 2 log cycles at equivalent radiation dose. During storage, a significant increase in both TPC and Y&MC was observed ($p < 0.05$) in control as well as radiation-treated samples. However, radiation processed samples at all doses had significantly ($p < 0.05$) lower microbial counts as compared to control throughout the storage period. At the end of storage period (28 d) TPC was > 9 log CFU/g for control samples while TPC for radiation-treated (2 kGy) samples was < 4 log CFU/g.

When ionizing radiation strikes bacteria and other microbes, its high energy breaks chemical bonds in molecules that are vital for cell growth and integrity. As a result, the microbes die, or no longer multiply to cause spoilage [150]. Radiation induced reduction of microbial load by 5-log cycle in french beans at a dose of 2 kGy [122], and a 2 log cycle reduction in radiation-treated celery (1.5 kGy) was observed [151]. Reductions of approximately 5.25 and 4.14 log CFU/g of a five-strain cocktail of *L. monocytogenes* were reported for cabbage and tomato, respectively, at a radiation dose of 1 kGy [88,152] reported that the initial bacterial load in control carrot samples (*Daucus carota*) was 6.3×10^2 CFU/g in control samples which increased to 6.5×10^5 CFU/g after 14 days of storage. A dose of 1 kGy reduced the bioload down to 12.0 CFU/g, with few colonies after 14 d storage. The samples receiving 2 kGy or higher doses were found to be completely free of bacteria during 14 days of refrigerated storage. Thus, the results obtained in the present study are in accordance with already published literature data.

It is proposed that elimination of spoilage bacteria can bring about enhanced growth of some pathogenic bacteria. Reports however document 4–5 log cycle reduction of L. monocytogenes in radiation processed sprouts [153] and watercress [154]. Thus pathogenic microbes were not monitored in the present study.

3.1.1.2. Color

The three Commission Internationale de l'Eclairage (CIE) co-ordinates "L*", "a*" and "b*" values were measured for all the three vegetables**.** "L*" value represents luminosity of the sample and varies from 0 (black) to 100 (white). Increase in luminosity can be correlated with the development of whiteness in the samples, while a decrease of luminosity can be an indication of browning appearance. In case of ash gourd and pumpkin, significant ($p \leq 0.05$) effect of radiation treatment and storage was observed in "L*" values, however, no significant ($p \leq 0.05$) change in "a*" and "b*" values was noticed. On the other hand, for processed drumstick samples, significant changes were noticed in "a*" values (+ red, - green) while "L*" and "b*" values were not affected. Therefore "L*" values for ash gourd and pumpkin, and "a*" values for RTC drumstick were analyzed with respect to radiation treatment and storage. Figure 15 depicts the effect of radiation treatment and storage on the color attributes of ash gourd, drumstick and pumpkin.

Ash gourd: The effect of radiation processing and storage duration on L* values is depicted in figure 15 (a). L* values continuously decreased with storage time in the control samples. Beyond 5 d, the control samples exhibited appreciable lowering in L* values and visual browning that increased during further storage. L* values also

decreased for samples irradiated at lower doses (0.5–1.5 kGy) after 8 d. It is interesting to note that the luminosity (L*) of the cut ash gourd irradiated at 2 and 2.5 kGy remained unchanged during storage and the visual quality was acceptable at the end of storage period (12 d).

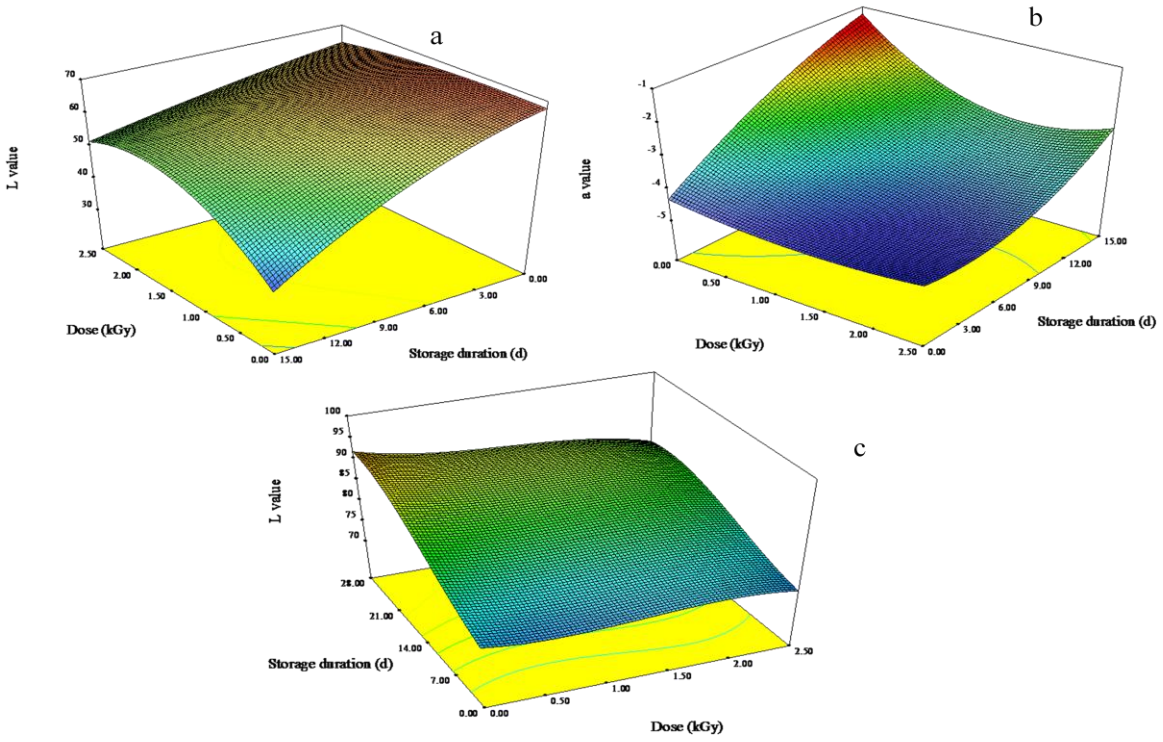


Figure 15. Effect of radiation treatment and storage on (a) L values of RTC ash gourd; (b) a values of RTC drumstick; (c) L values of RTC pumpkin.

Radiation induced inhibition in surface browning has been documented by [155] in fresh-cut vegetables. Decrease in surface browning of lettuce with increasing radiation dose was also reported by Fan et al [94, 156]. A negative correlation between browning intensity and lightness (L* value) has been documented [157]. Polyphenoloxidase (PPO) is a key enzyme responsible for browning in fruits and vegetables, which catalyzes

oxidation of phenolic compounds, resulting in formation of colored melanins. Radiation treatment might alter the reactions catalyzed by these enzymes, thus limiting the development of brown pigments.

Drumstick: On day zero, no significant ($p \leq 0.05$) effect of radiation processing was observed on the "a*" values of cut drumstick samples. A positive value of 'a*' indicates increased perception of redness, while negative values indicate greenness. During storage, "a*" (-ive) values decreased with increasing storage period in control samples (Figure 15 b), indicating a reduction in the greenness. Reduction of greenness might be due to degradation of pigments during storage. Higher "a*" values (-ive) were obtained for radiation treated samples as compared to control samples during the entire storage duration indicating better retention of greenness throughout the intended storage period.

Pumpkin: The effect of radiation treatments and storage on L values of RTC pumpkin is depicted in Figure 15 (c). Radiation processing had no significant ($p < 0.05$) effect on L* values. A significant ($p < 0.05$) increase in L* values was however observed for both control and radiation-treated samples with increasing storage duration (0 to 28 d). Moreover, during storage the change in L* values for radiation processed samples was significantly lower as compared to control. The L* value on day zero was 75.0 ± 3.23 which increased to 96.0 ± 5.48 and 83.0 ± 4.16 on day 28 for control and irradiated (2 kGy) samples, respectively. An increase in L* values signifies a decreased colour intensity of control pumpkin samples. A similar trend was reported earlier in *Citrus clementina* [158] and french beans [122].

In general, during extended storage, fading or decreased color intensity has been reported in various fresh-cut products. Degradation of pigments during storage resulting in fading of the color has been suggested as the probable reason for this observation [122]. Reduced change in color attribute (L*, a* or b* value) of radiation treated samples during storage as compared to control might be due to lower rate of respiration and physiological changes, thus resulting in better retention of pigments and higher color intensity during storage.

3.1.1.3. Texture

Minimally processed vegetables that maintain firm, crunchy texture are highly desirable because consumers associate these textures with freshness and wholesomeness [159]. During mechanical operations, cut surfaces are damaged, releasing enzymes which spread through the tissue and come into contact with their substrates. The softening of fresh-cut fruit is mainly due to the enzymatic degradation of the cell wall, which is mainly composed of cellulose, hemicellulose and pectins. The loss of desirable texture in fresh-cut products is a major problem. It was therefore of interest to study the effect of radiation processing and storage on firmness of the RTC vegetables.

Ash gourd: A significant ($p \leq 0.05$) effect of storage time and radiation processing on the firmness of samples was observed (Figure 16 a). Irradiation resulted in decreased firmness ($p \leq 0.05$) at all the doses studied on day zero. During storage, continuous decrease in firmness was observed in control and samples irradiated at lower doses (0.5–1.5 kGy). However, in samples irradiated at 2 and 2.5 kGy, the firmness quality was not significantly ($p \leq 0.05$) affected during storage.

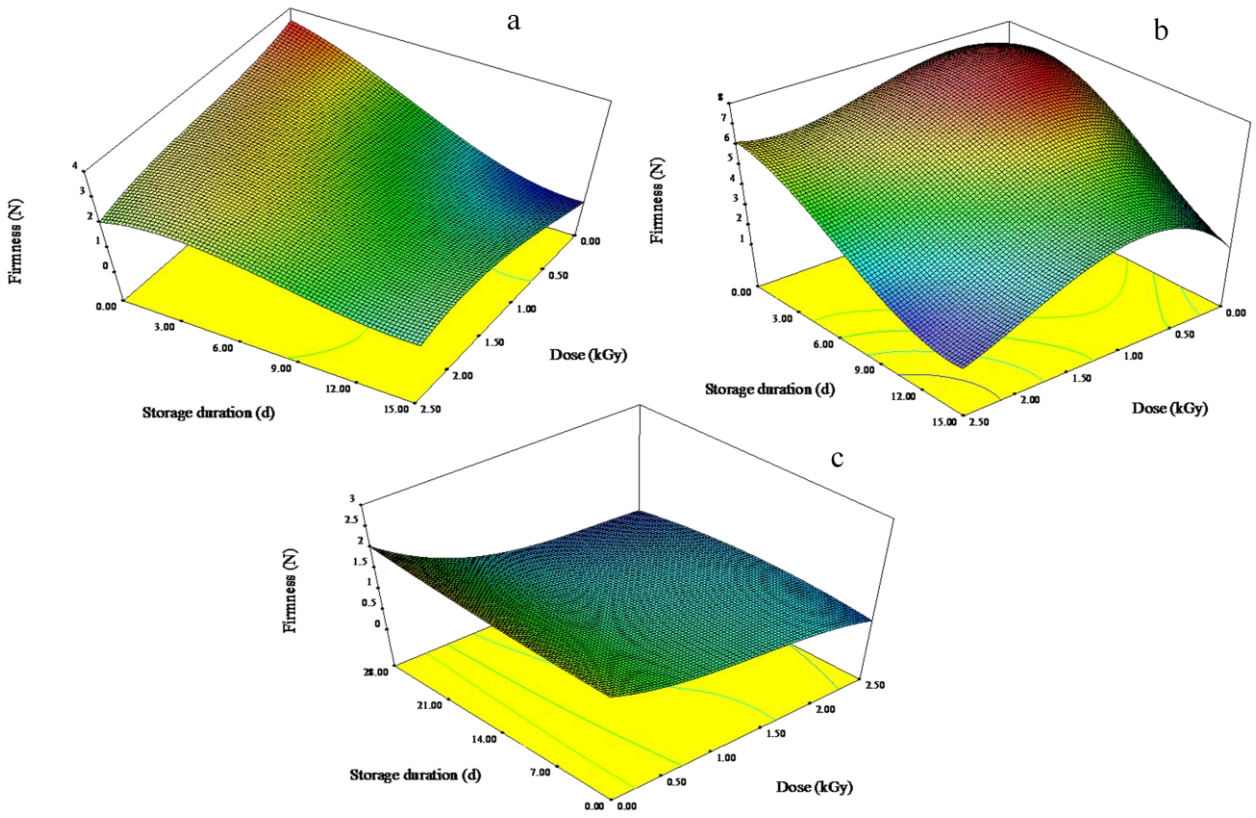


Figure 16. Effect of radiation treatment and storage on firmness of (a) Ash gourd; (b) Drumstick; (c) Pumpkin.

Drumstick: Effect of radiation processing and storage duration on the firmness of cut drumstick is depicted in Figure 16 (b). A dose dependent decrease in firmness was observed in radiation processed samples on day zero. In non-irradiated and samples irradiated at 0.5 kGy, firmness decreased continuously during the storage. At 1 kGy, the firmness was not significantly affected as a result of storage. At doses > 1.0 kGy, significant softening was observed immediately after irradiation which increased further during storage.

Pumpkin: A radiation dose-dependent reduction in firmness of RTC pumpkin cubes was observed on day zero with a 50 % reduction at 2.0 kGy (Figure 16 c). Interestingly, an

increase in firmness of control samples was observed during storage (1.5 ± 0.2 N at 0 d to 2.1 ± 0.2 N at 28 d). However, no significant increase in firmness during storage was noticed in radiation-treated samples. This could possibly be due to the lower respiration rate in radiation treated samples resulting in decreased water loss and subsequently better maintenance of texture. The firmness of the developed radiation-treated product was therefore maintained during the entire intended storage while control samples exhibited increased firmness after seven days of storage.

Radiation processing is known to induce softening immediately after treatment. Such observations have been previously attributed to radiation-induced hydrolysis of pectin and other cell wall components such as cellulose and hemicellulose [160]. No significant changes in texture during storage have been reported in various radiation-treated fresh-cut produce such as celery, green leaf lettuce, iceberg lettuce, parsley and red leaf lettuce by Fan & Sokorai, 2008 [12]. The better retention of texture of radiation processed samples during storage could possibly be due to lower rate of respiration in plant produce which results in decreased water loss and subsequently better firmness [161].

On the other hand, increased firmness in fresh-cut tissues during storage has also been reported earlier in some cases such as ready-to-use carrots, where a significant increase in firmness was observed during storage [162]. Lignification was cited as the probable reason by these researchers. Due to removal of outer epidermal layer to form peeled ready-to-use product, physiological changes occur in the vegetable to initiate the production of a new protective layer of lignin, giving a stiff aspect to the fresh-cut vegetable and requiring greater force to break the plant tissue. Also water loss in fresh-cut

produce is known to result in drying of the outer layers and hence requiring more force to pierce into the tissue [162].

3.1.1.4. Sensory acceptability

Consumer integrates all sensory inputs - appearance, texture, off-flavours and odours - into a final judgement of the acceptability of that fruit or vegetable. In order to get an appropriate implementation of a treatment for preservation or improvement in shelf life, sensory evaluations need to be carried out to ensure that the perceived quality of a determined product is not negatively affected. The sensory analysis was therefore conducted to study the effect of radiation treatment and storage on consumer acceptability.

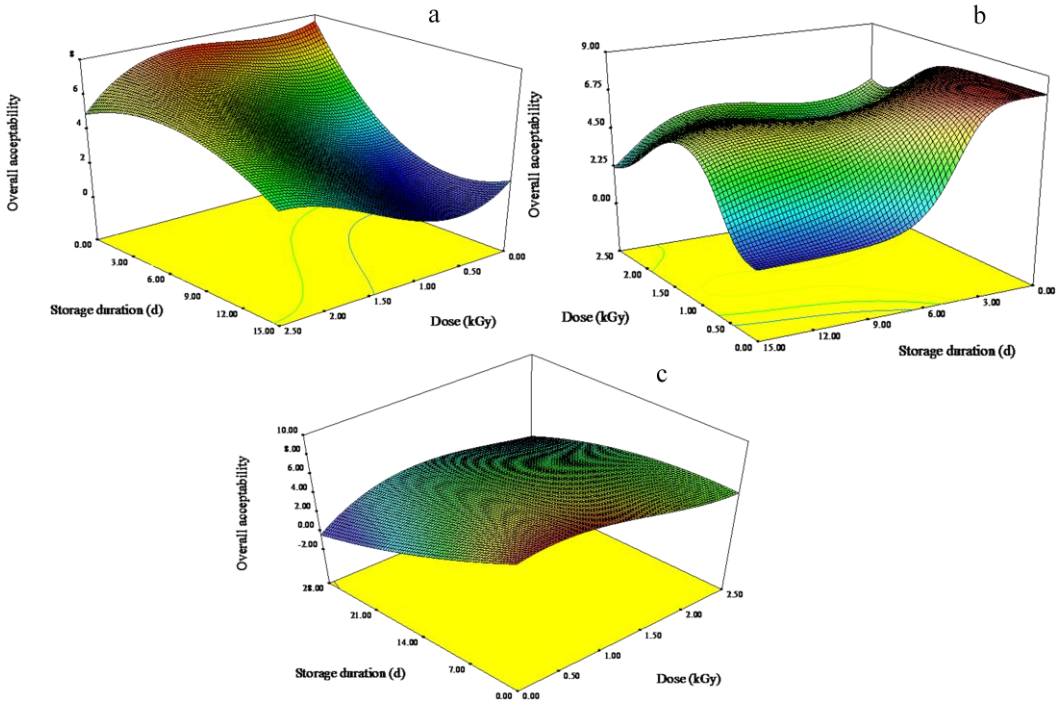


Figure 17. Effect of radiation treatment and storage on the overall sensory acceptability of (a) Ash gourd; (b) Drumstick; (c) Pumpkin.

Ash gourd: The results of the hedonic test for the overall acceptability of the product are depicted in Figure 17 a. No significant difference ($p \leq 0.05$) in hedonic scores of control and irradiated samples up to a dose of 1.5 kGy was noted after irradiation on day zero. Samples irradiated at 2 kGy and above exhibited softening in texture, resulting in lower scores, however it was statistically insignificant. The sensory quality of control samples was found to deteriorate within 5 d of storage. Between radiation doses 0.5–1.5 kGy, the acceptability of the samples decreased with storage. However, at 2 and 2.5 kGy, it remained unchanged during entire storage period. Interestingly, the acceptability of samples at 2 kGy was higher than that of 2.5 kGy at all stages of storage. This could be due to better retention of texture in samples irradiated at 2 kGy as compared to that of 2.5 kGy. These samples (2 kGy) also scored better in aroma and taste attributes making them more acceptable compared to the other samples. Hedonic analysis thus indicated that radiation processed samples at a dose of 2 kGy had the highest scores throughout the storage period.

Drumstick: Figure 17 b shows the results of hedonic testing for overall acceptability of control and radiation processed drumstick samples. The overall acceptability of the samples was unaffected up to a radiation dose of 1.0 kGy, while it decreased at doses > 1.0 kGy on day zero. The hedonic scores obtained for control and samples subjected to radiation doses of 0.5 and 1.0 kGy were 8.0 ± 0.3, 7.5 ± 0.5 and 7.0 ± 0.5 respectively, while that for irradiated at 1.5, 2.0 and 2.5 kGy lied between 5 and 6. This might be attributed to decreased firmness of the samples at higher doses. The acceptability of control and samples irradiated at 0.5 kGy decreased drastically from day 5 onwards. This

may be attributed to reduced aroma and texture scores obtained. The samples irradiated at 1 kGy exhibited excellent sensory quality during storage period up to 12 d with a sensory score of 7 ± 0.5 for overall acceptability; however, the sensory score decreased to < 4 on day 15.

Pumpkin: The hedonic scores plotted against radiation doses and storage duration is shown in Figure 17 c. On day zero, the effect of radiation dose up to 1 kGy on the hedonic scores was insignificant ($p < 0.05$). The values obtained were, respectively, 8.0 ± 0.7, 7.5 ± 0.5 and 7.0 ± 0.25 for control and samples subjected to radiation doses of 0.5 and 1.0 kGy. Sensory scores for samples irradiated at higher doses (beyond 1 kGy) were significantly lower as compared to control on day zero. Scores for these samples were between 4.5 and 5.5. This might be attributed to decreased firmness perceived by sensory panel at these doses. Decrease in firmness is also evident from the instrumental data for firmness (Figure 16 c). The acceptability of control and irradiated samples (0.5 kGy) decreased drastically from day 14 onwards. This could be explained on the basis of reduced aroma notes perceived by sensory panel and harder texture of the samples towards the end of storage resulting in lower scores. The samples irradiated at 1 kGy exhibited excellent sensory quality during storage period up to 21 d with a sensory score of 6 ± 0.5 for overall acceptability; however, the sensory score decreased to < 3 on day 28. Significantly higher aroma scores for radiation processed carrots, cilantro, green onions, parsley and red lettuce after 14 days of storage have been reported earlier [12].

3.1.1.5. Optimization and verification of results

The processing factors were optimized for achieving maximum shelf-life of the RTC products with acceptable sensory and microbial quality. Apart from this, the optimized levels of the variables and the importance of the responses were also taken into account. Criteria set for optimization and solutions obtained for the individual vegetable product are depicted in Table 26. The criteria decided aimed at maximizing shelf life of product with mesophillic load of < 5 log CFU/g, minimum change in color & texture and an overall acceptability score of greater than 5.

Table 26. Criteria for various factors and responses for process optimization and corresponding optimized solutions obtained

Name	Criteria 1	Criteria 2	Solution A	Solution B
Ash gourd				
A:Dose (kGy)	minimize	minimize	2.34	1.95
B:Storage time (d)	maximize (8-15)	maximize (5-12)	14	12
TPC (Log CFU/g)	minimize (<5)	is in range (<6)	3.95^{a}; $(3.76 \pm 0.58)^{b}$	3.92^{a}; $(3.86 \pm 0.41)^{b}$
Y&M (Log CFU/g)	minimize	minimize	2.35^{a}; $(2.28 \pm 0.21)^{b}$	1.60^{a}; $(1.52 \pm 0.22)^{b}$
Color (L)	In range (39.0-68.0)	In range (45- 68)	53.15^{a}; $(57.56 \pm 2.54)^{b}$	62.09^{a}; $(61.29 \pm 4.67)^{b}$
Firmness (N)	maximize(1.6-3.5)	in range (1.0-3.5)	1.75^{a}; $(1.84 \pm 0.50)^{b}$	2.32^{a}; $(2.15 \pm 0.35)^{b}$
Overall acceptability (Hedonic)	In range (5-7)	≥ 5	5.52^{a}; $(5.65 \pm 0.25)^{b}$	5.38^{a}; $(5.21 \pm 0.25)^{b}$
Drumstick				
A:Dose (kGy)	minimize	minimize	1.5	0.98
B:Storage time (d)	maximize (5-12)	maximize (5-15)	7	12
TPC (Log CFU/g)	Minimize (<5)	In range (<6)	3.33^{a}; $(3.52 \pm 0.52)^{b}$	4.99^{a};$(5.10 \pm 0.45)^{b}$
Y&M (Log CFU /g)	minimize	minimize	1.33^{a}; $(1.62 \pm 0.42)^{b}$	2.28^{a}; $(2.12 \pm 0.54)^{b}$
Color (a)	In range (-4.5- -1.5)	In range (-4.5- -1.5)	-4.32^{a}; $(-4.45 \pm 0.98)^{b}$	-3.25^{a}; $(-3.14 \pm 0.41)^{b}$
Firmness (N)	In range (4.41-7.45)	maximize(0.6-1.0)	4.26^{a}; $(4.59 \pm 1.55)^{b}$	3.65^{a}; $(3.88 \pm 1.25)^{b}$
Overall acceptability (Hedonic)	≥ 5	≥ 5	5.36^{a}; $(5.3 \pm 0.4)^{b}$	6.00^{a}; $(5.5 \pm 0.6)^{b}$

Pumpkin				
A:Dose (kGy)	minimize	minimize	1.2	1.0
B:Storage time (d)	maximize (18-21)	maximize (14-21)	21	21
TPC (Log CFU/g)	minimize (< 5)	is in range (< 6)	3.79[a];(4.12 ± 0.45)[b]	4.35[a]; (4.92 ± 0.52)[b]
Y&M (Log CFU /g)	minimize	minimize	2.91[a]; (3.12 ± 0.54)[b]	3.02[a]; (3.62 ± 0.42)[b]
Color (L)	minimize	minimize	87.11[a]; (82.14 ± 5.41)[b]	87.65[a]; (90.14 ± 4.98)[b]
Firmness (N)	is in range (0.6-1.0)	Maximize (>0.8 <1.7)	0.96[a]; (0.89 ± 0.12)[b]	1.04[a]; (0.99 ± 0.21)[b]
Overall acceptability (Hedonic)	In range (5-8)	≥ 5	5.26[a]; (5.5 ± 0.6)[b]	5.18[a]; (5.3 ± 0.4)[b]

[a]Predicted values for solutions.
[b]Actual values.

Two solutions were obtained for each vegetable depending on the criteria decided. Solution A and B were obtained for criteria 1 and 2 respectively. For ash gourd Solution A suggested a radiation dose of 2.34 kGy for a shelf life of 14 d while a shelf life of 12 d was suggested at 2 kGy dose in solution B. For RTC drumstick, solutions A and B suggested a radiation dose of 1.5 and 1.0 kGy for a shelf life of 7 and 12 d respectively. In case of pumpkin, Solution A and B suggested a radiation dose of 1.2 and 1 kGy, respectively, with a storage period of 21 d.

Models generated were validated using both the solutions obtained for each vegetable separately. For the validation experiments, fresh samples of ash gourd, drumstick and pumpkin were cut, packaged and irradiated at doses suggested in solutions obtained (2.3 and 2.0 kGy for ash gourd; 1.5 and 1.0 kGy for drumstick; 1.2 and 1.0 kGy for pumpkin). They were subsequently analyzed for microbial quality (TPC and Y&MC), color, texture and sensory acceptability during the intended storage period as obtained in solutions A and B. A good agreement of actual and predicted values indicated the suitability of the models (Table 26).

Both the solutions obtained for the three vegetables demonstrated a microbial load < 5 log CFU/g and acceptable sensory quality (overall acceptability above 5). The solutions corresponding to the lower radiation doses with acceptable microbial and sensory quality were selected for each vegetable. Thus an optimum dose of 2 kGy and shelf life of 12 d for ash gourd, and a dose of 1 kGy with a shelf life of 12 and 21 d for drumstick and pumpkin respectively, was chosen. The products processed at these radiation doses were further analyzed for the nutritional quality in terms of radical scavenging activity, total phenolic and flavonoid content, vitamin C content, head space gaseous composition and quantitative descriptive sensory analysis (QDA). Bioactive constituents were also analyzed for the developed products at the optimized dose.

3.2. Characterization of the developed RTC products

Figure 18 presents the control and radiation processed products of RTC ash gourd, drumstick and pumpkin.

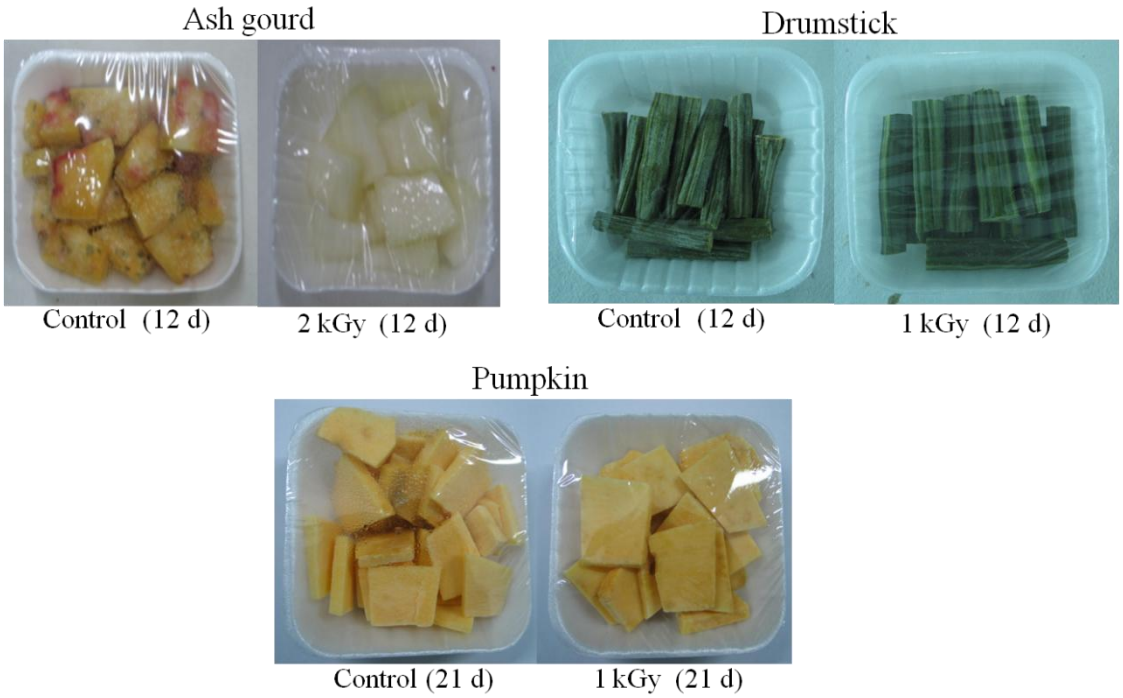


Figure 18. Control and radiation processed (at optimum dose) RTC products for ash gourd, drumstick and pumpkin at the end of intended storage period.

For ash gourd, optimum solution obtained was a shelf life of 12 d at a radiation dose of 2 kGy, while for drumstick and pumpkin the optimum parameters were a shelf life of 12 and 21 d respectively at a radiation dose of 1 kGy. The RTC products developed for the selected vegetables were further characterized for sensory quality by quantitative descriptive analysis (QDA), nutritional quality in terms of radical scavenging activity, total phenolic and flavonoid content, vitamin C content, head space gaseous composition inside the packages and aroma quality at the optimized conditions.

3.2.1. Sensory quality evaluation by Quantitative Descriptive Analysis (QDA)

The fresh control samples as well as those treated with optimum radiation dose and stored for intended duration were evaluated for sensory attributes by QDA. Independent training sessions were carried out to familiarize the sensory panel for assessing the product quality and generate attributes for descriptive analysis.

Ash gourd: The sensory attributes evaluated for RTC ash gourd were texture, color, aroma (ash gourd, irradiated, buttery and musty) and taste (ash gourd, irradiated). The control samples at the end of storage (12 d) were not evaluated as they were spoiled. The data obtained are represented by a spider diagram (Figure 19). The texture of the processed product was not significantly affected after radiation processing on day zero; however, softening was observed on the twelfth day of storage, which is in agreement with the scores obtained on the hedonic scale. At the end of storage period (12 d), the radiation processed product had slightly lower scores for attributes like buttery and ash gourd aroma. No difference in taste was observed between processed products and fresh control samples. QDA results thus indicate that radiation processed (2 kGy) ash gourd had acceptable aroma, taste and textural properties during an extended storage period of 12 d.

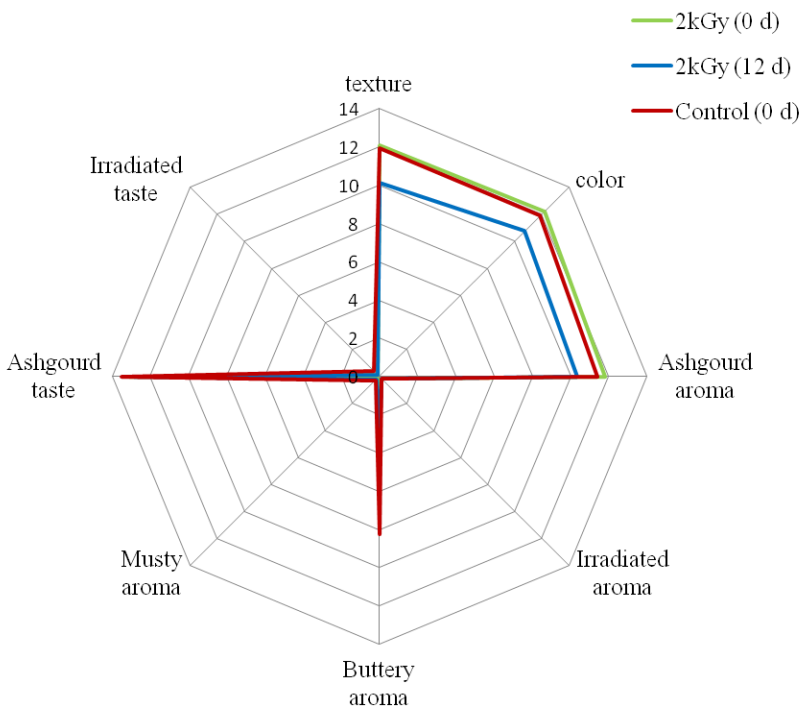


Figure 19. Quantitative descriptive analysis of fresh control and radiation-processed ash gourd samples at day zero and twelve of storage.

Drumstick: The data obtained from QDA of RTC drumstick samples is represented as a spider graph in Figure 20. The sensory attributes studied were texture, color (green, brown), aroma (drumstick, green, irradiated, and musty) and taste (drumstick, sweet and irradiated). Control samples on day 12 were not evaluated for taste as they exhibited visible fungal growth. On day zero no significant effect of irradiation was observed on the attributes studied. At the end of intended storage period (12 d), the irradiated samples exhibited slightly lower but statistically insignificant scores for texture and color. However, no significant effect of storage was seen in the other attributes of radiation processed samples as compared to fresh-cut drumstick samples of day zero. The results

indicated that radiation treated (1 kGy) samples had acceptable sensorial properties during an extended storage period of 12 d.

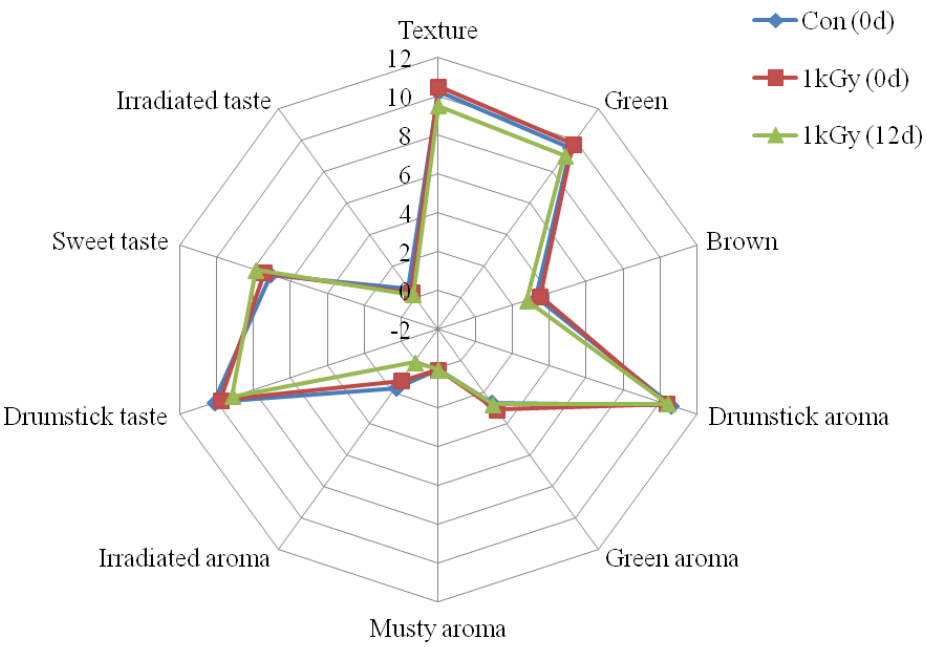


Figure 20. Quantitative descriptive analysis of fresh control and radiation-processed drumstick samples at day zero and twelve of storage.

Pumpkin: The sensory scores as obtained by QDA of pumpkin are shown pictorially by a spider graph in Figure 21. Control samples on day 21 were not evaluated for taste as they were spoiled. After 21 d of storage, the irradiated (1 kGy) product had marginally lower scores for texture and color. This is also evident from the instrumental data for color and texture. However, no significant difference in taste and aroma were noted between radiation-treated products at day 21 and fresh control samples. No irradiated off flavor or taste was perceived by the assessors. Similar results have been reported in various

radiation-treated minimally processed vegetables [86]. QDA results thus established that radiation processed (1 kGy) pumpkin had acceptable aroma, taste and textural properties during an extended storage period of 21 d.

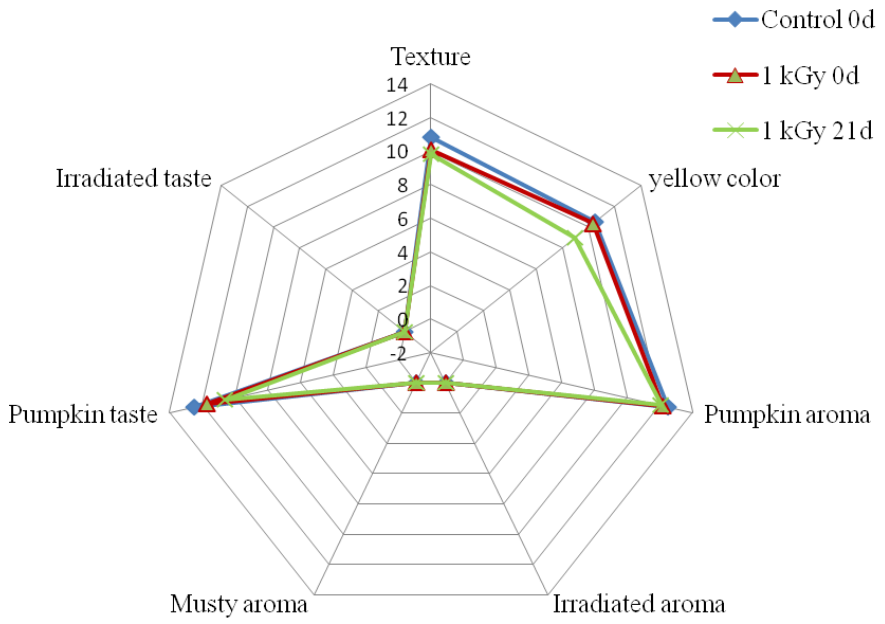


Figure 21. Quantitative descriptive analysis of fresh control and radiation-processed pumpkin samples at day zero and 21 of storage.

3.2.2. Nutritional quality

The fresh-cut products are claimed to be convenient and healthy alternatives to fulfill the dietary needs for fresh food. However, the changes that happen during harvesting, handling and processing can affect antioxidant status. Lindley (1998) [163] and Klein (1987) [164] reviewed the nutritional consequences of minimal processing on fruit and vegetables and concluded that conditions that maintain desirable sensory characteristics

will also preserve nutrients. Levels of ascorbic acid, carotenoids or polyphenols can reflect the variations in antioxidant capacity of fruit and vegetables [164]. The antioxidant potential in the form DPPH radical scavenging activity, and the principal components which could contribute towards the antioxidant potential such as phenolic, flavonoid and vitamin C contents were therefore analyzed in response to radiation treatment during storage.

3.2.2.1. DPPH Radical scavenging activity

Ash gourd: Total antioxidant activity of ash gourd was found to be 169.9 ± 11.3 mg kg^{-1} of fresh weight. In control samples, the radical scavenging activity decreased on day 5 and then remained unchanged on subsequent storage. The activity increased significantly ($p \leq 0.05$) in response to radiation treatment compared to the corresponding control at all stages of storage (Figure 22 A). A lowering in antioxidant activity was, however, noted on storage of samples beyond 8 d in the irradiated samples.

Drumstick: The radical scavenging activity of drumstick was found to be 125.56 ± 8.6 mg kg^{-1} of fresh weight. Radiation treatment increased the antioxidant potential compared to control samples. This increase was, however, not statistically significant ($p \leq 0.05$). An appreciable decrease in the activity was observed during storage in control as well as irradiated samples (Figure 22 B). A decrease of 39 % and 43 % in activity was observed in control and radiation processed samples on day 5. Beyond 5 d, activity decreased continuously in control samples, while it remained unaffected in radiation processed samples. The radiation treated samples (1 kGy) were therefore better than the

corresponding control in terms of DPPH scavenging activity at the end of storage period (12 d).

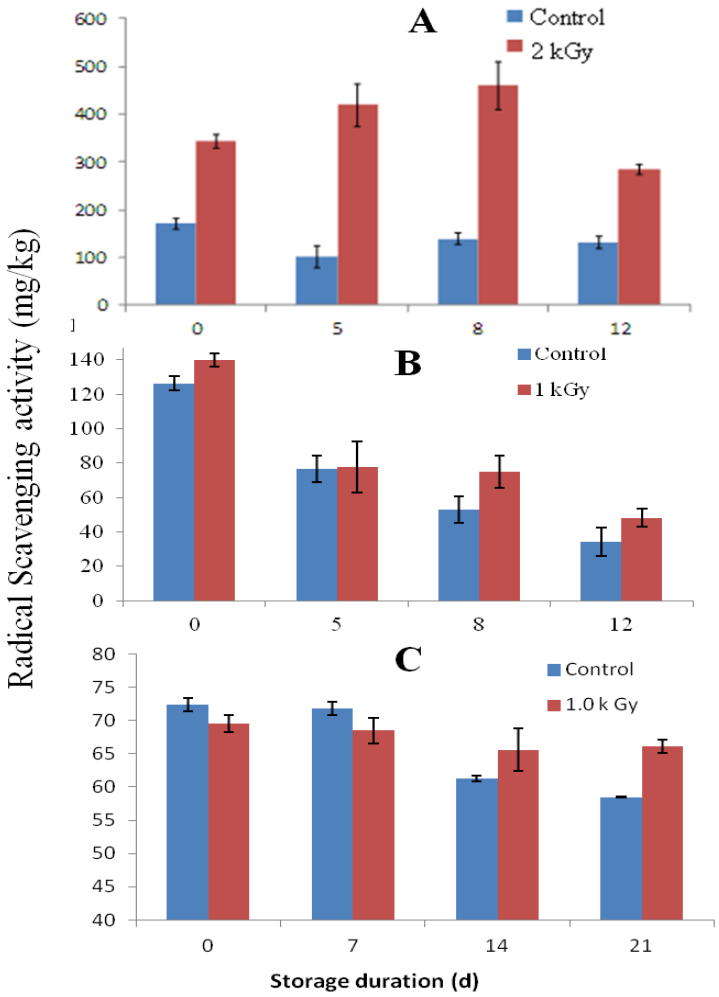


Figure 22: Variation in DPPH radical scavenging capacity of control and radiation processed RTC vegetables with storage. (A) Ash gourd, (B) Drumstick, (C) Pumpkin

Pumpkin: An appreciable radical scavenging activity in pumpkin has been reported earlier [165]. In the present study, the antioxidant capacity in pumpkin was found to be 72 mg/kg of pumpkin. No statistically significant ($p < 0.05$) change was observed in

antioxidant activity due to radiation processing (1 kGy) at zero day (Figure 22 C). During storage, the antioxidant activity was unchanged in control samples up to 7 days, while a 20% decrease was observed on day 21. On the other hand, irradiated samples had no significant ($p<0.05$) change in the activity during the intended storage period of 21 d.

Various studies are available in literature reporting changes in the antioxidant potential of fresh-cut vegetables as a result of processing. A decrease in the antioxidant activity after processing is reported for fresh-cut spinach [166] and mandarin [167], while, wounding is known to increase the antioxidant activity of Iceberg and Romaine lettuce [168]. It is known that irradiation generates free radicals that may act as stress signals and trigger stress responses in vegetables, resulting in increased antioxidant synthesis [94]. It has been also proposed that gamma radiation is capable of breaking the chemical bonds of polyphenols, resulting in the release of low molecular weight soluble phenols, leading to an increase in antioxidant rich phenolics [92]. These could possibly be some of the reasons for observed increase in radical scavenging activity in irradiated ash gourd and drumstick. However, during storage, the activity declined which might be due to degradation of antioxidants as a result of oxidation on prolonged storage.

3.2.2.2. Total phenolic and flavonoid content

Phenolic compounds including flavonoids are important bioactive compounds that have the ability to reduce free radical formation and to scavenge free radicals. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and light screener [43]. The total phenolic and flavonoid content as estimated in ash gourd, drumstick and pumpkin is shown graphically in Figure 23.

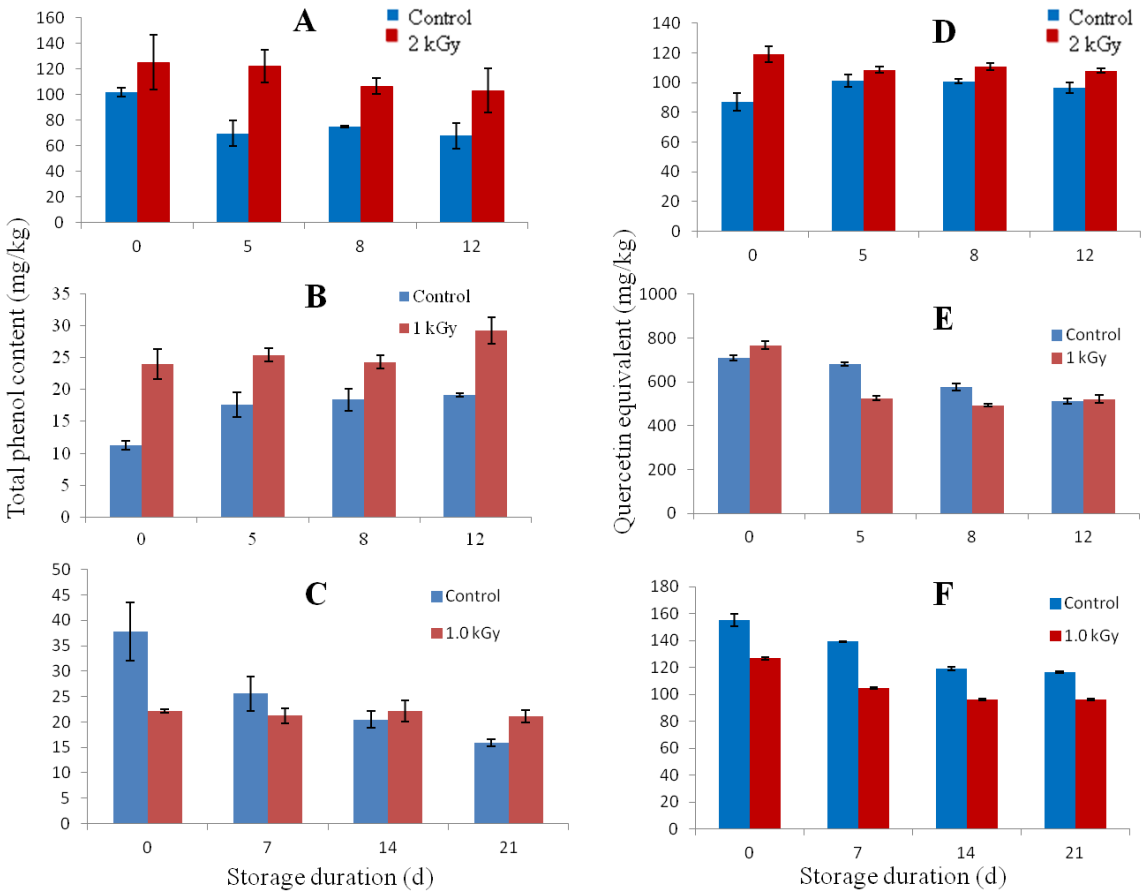


Figure 23: Variation in total phenolic and flavonoid content in ash gourd (A & D); drumstick (B & E) and pumpkin (C & F) due to radiation processing and storage.

Ash gourd: Total phenolic content was found to be 102.08 ± 3.44 mg kg^{-1} of ash gourd in the present study. Changes in total phenolic and flavonoid content with radiation processing and storage are depicted in Figure 23 A & D respectively. Radiation treatment resulted in a significant ($p \leq 0.05$) increase in the phenolic and flavonoid content on day zero, and it remained unaffected during the entire storage period. In control samples, the total phenolic content decreased significantly ($p \leq 0.05$) during an initial storage period of 5 d, while a marginal but statistically insignificant increase was observed in the flavonoid

content on day 5. The phenolic as well as flavonoid contents remained unchanged during the remaining storage period. Total phenolic and flavonoid contents in radiation treated samples were found to be significantly higher ($p < 0.05$) than the corresponding controls on all storage days studied. Thus the nutritional quality of the developed radiation processed product was better at the end of storage period (12 d).

Drumstick: A significant effect of radiation processing was observed on the total phenolic content of RTC drumstick (Figure 23 B). The phenolic content in the radiation processed samples (24 ± 2.35 mg/kg) was twice that of that of the control (11.24 ± 0.69 mg/kg) on day zero, which remained unchanged during storage duration. Irradiated samples exhibited significantly ($p < 0.05$) higher phenolic content than corresponding controls on all days of storage.

At day zero, radiation processing caused a marginal increase in flavonoid content, however, the increase was not statistically significant (Figure 23 E). A decrease in the content was observed during storage in control as well as radiation processed samples. The contents in control and irradiated samples were comparable at the end of storage period (12 d).

Pumpkin: In **c**ontrast to the ash gourd and drumstick, a significant ($p < 0.05$) decrease in the total phenolic and flavonoid content was observed in response to radiation treatment (Figure 23 C & F). Gamma radiation and UV-C induced decrease in antioxidant compounds such as phenolics and flavonoids has been reported previously in various food commodities [169, 170]. During storage, the contents decreased in control samples, however it was unchanged in the radiation processed samples. A decrease in the flavonoid

content during storage in orange and grapefruit juices has been reported earlier [171]. The decrease was explained by degradation or oxidation of sensitive phenolic constituents during extended storage.

Radiation induced increase in the flavonoid content was reported earlier in mushrooms [172] and bitter gourd at radiation doses ranging from 0.25 – 1.0 kGy. Accumulation of phenolic compounds and flavonoids in response to radiation as a defense mechanism in plants has been reported. The increase has been attributed to the phenylalanine ammonia lyase activity which is one of the key enzymes in the synthesis of phenolic compounds in plant tissues [173]. Increase in phenolic compounds in irradiated plant produce has also been attributed to depolymerization and dissolution of cell wall polysaccharides which facilitated higher extractability [85]. The results obtained in the present study are in agreement with those reported by other authors for minimally processed vegetables [173, 174]. The trends obtained for the variation in phenolic contents in response to radiation treatment and storage were similar to that obtained for DPPH radical scavenging activity. It indicates that phenolic compounds present in ash gourd, drumstick and pumpkin play an important role towards their radical scavenging potential.

3.2.2.3. Vitamin C content

Vitamin C is one of the most sensitive vitamins, being degraded quickly on exposure to heat, light, oxygen, processing and storage. It works as an antioxidant by protecting the body against oxidative stress and also as a cofactor in several vital enzymatic reactions [175]. It was therefore of interest to monitor the effect of radiation processing and storage on the content of vitamin C in the developed RTC products.

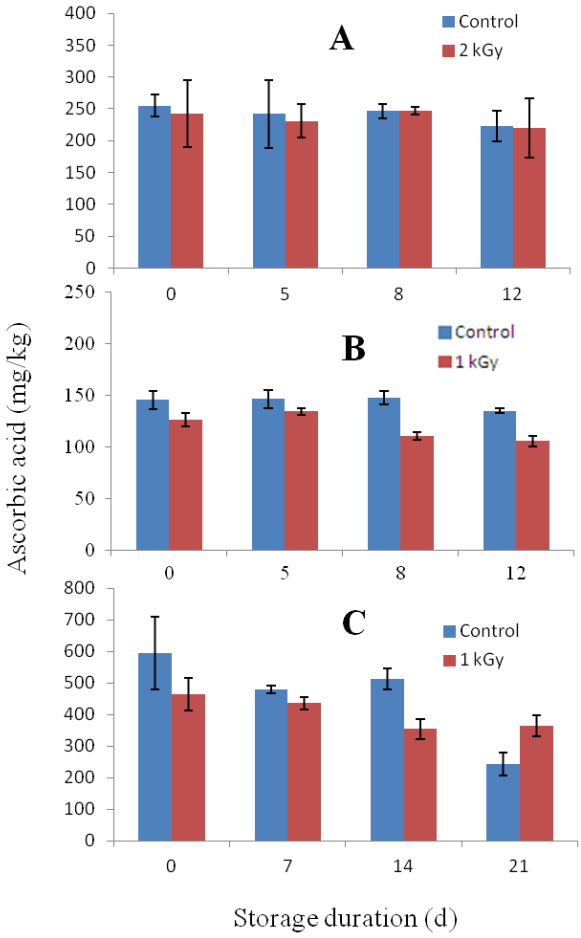


Figure 24. Effect of radiation processing and storage on ascorbic acid content of (A) Ash gourd; (B) Drumstick; and (C) Pumpkin

Ash gourd: The amount of vitamin C in the present study was found to be 250 mg/kg of ash gourd. The amount estimated is in agreement with the previous reports available [135, 100]. Vitamin C content of RTC ash gourd remained unaffected in response to radiation treatment. No significant change ($p \leq 0.5$) in the vitamin C content was observed during storage in both the control (255–222 mg/kg) and radiation processed ash gourd (242–219 mg/kg) in the present study (Figure 24 A). Pandey et al. have, however, reported a

decreasing trend in the total vitamin C content in intact ash gourd during storage [176]. This different observation could be due to the methodology adopted wherein the total ascorbate content was measured in its reduced form in the present study.

Drumstick: The content of vitamin C in fresh control drumstick samples (145.50 ± 9.17 mg/kg) was found to be in agreement with that reported in literature (141 mg/kg of edible portion) [112]. A 13 % decrease in the content of vitamin C was observed immediately after radiation treatment (1 kGy) (Figure 24 B). Storage resulted in a marginal decrease in the vitamin C content of control as well as irradiated samples, however, this decrease was not statistically significant ($p < 0.05$).

Pumpkin: Fresh control samples of pumpkin had vitamin C content of 600 mg/kg. A 27 % decline in vitamin C content was observed immediately after radiation processing (1 kGy), which did not change significantly ($p < 0.05$) during storage (Figure 24 C). However, in the control pumpkin samples, a 60 % decrease in vitamin C content was observed on day 21. At the end of the storage period, the vitamin C content of radiation-treated samples (0.35 mg/kg) was significantly higher ($p<0.05$) than that of control samples (0.23 mg/kg), making its nutritional quality better than control samples on day 21.

Losses in vitamin C content during storage have been known in different fruits and vegetables [177]. Better retention of vitamin C during extended storage in radiation processed sample has been observed earlier in different vegetables [12]. Thus, results obtained in the present study are in agreement with the previous reports available in literature.

3.2.3. Headspace gaseous composition

Minimally processed vegetables are living tissues. Damaged plant tissues exhibit an increase in respiratory rate. It has been reported that tissues with high respiratory rates have shorter postharvest life [161]. Headspace gas composition inside the packages plays an important role in controlling the nature and content of microbial growth. The content of O_2 and CO_2 was therefore monitored for both the control and radiation processed samples in all the three RTC vegetable packages at the optimized process parameters.

Ash gourd: Significant ($p \leq 0.05$) effects of irradiation and storage time were observed for both gases (O_2 and CO_2) in the packages (Figure 25A). In the control samples, a continuous decrease in O_2 and an increase in CO_2 were observed during storage. Irradiated samples had a higher CO_2 concentration compared to the control till 3 d of storage. During storage, the CO_2 concentration inside the package decreased gradually, while a corresponding increase in O_2 concentration was observed in the radiation processed samples until the fifth day. However, from day five onwards it remained constant for the intended duration of storage. At the end of storage, the gaseous composition was 13 % O_2 and 8.8 % CO_2 for control, and 19 % O_2 and 2.8 % CO_2 for the irradiated samples.

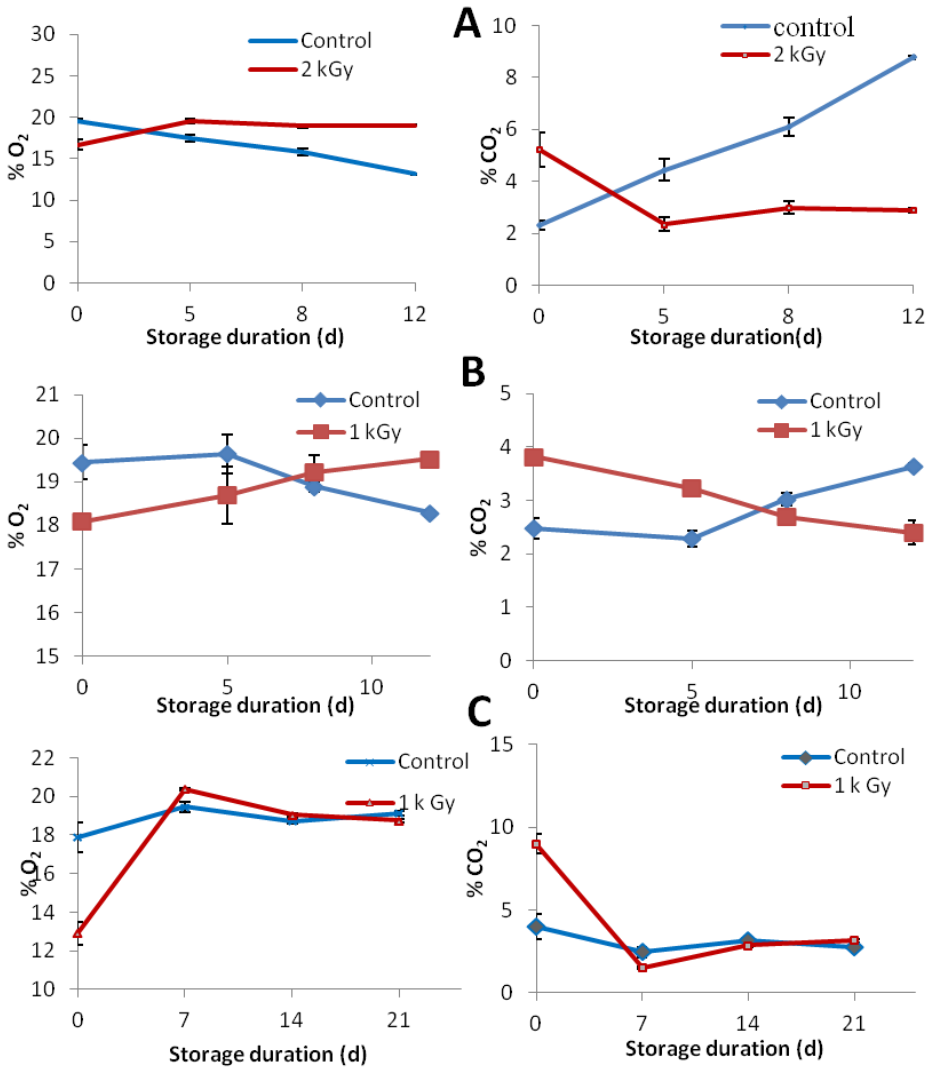


Figure 25. Headspace composition in control and radiation processed RTC (A) Ash gourd (B) Drumstick and (C) Pumpkin packages during storage.

Drumstick: O_2 and CO_2 contents inside the packages are depicted in Figure 25 B. Radiation processing resulted in significant ($p < 0.05$) decrease in O_2 and increase in CO_2 content immediately after treatment. The CO_2 content of radiation processed samples was higher than the control till day 7. However, during further storage, the CO_2 content

decreased in radiation processed samples and then remained constant, achieving equilibrium with the atmosphere. At the end of storage period (12 d), the gaseous composition for control and irradiated samples was 18.28 % O_2, 3.69 CO_2 and 19.5 % O_2, 2.39 % CO_2 respectively.

Pumpkin: Immediately after radiation treatment, packages of irradiated pumpkin had significantly ($p < 0.05$) lower O_2 and higher CO_2 levels (12.91% O_2 and 8.59% CO_2) than the controls (17.88 % O_2 and 3.62% CO_2) as depicted in figure 25 C. After 7 days of storage O_2 and CO_2 content in packages subjected to radiation processing were 19 and 2.5 %, respectively. Thus an increase in oxygen concentration as compared to zero day was observed, which remained constant for the remaining storage duration.

The continuous decrease in O_2 and increase in CO_2 observed during storage in control samples could be due to product respiration. A similar effect was observed in the case of cantaloupe and honey dew subjected to passive MAP [178]. Radiation induced decrease in O_2 and increase in the content of CO_2 was noted in all the three products immediately after radiation treatment. A similar effect has been demonstrated in broccoli and mushrooms where increase in metabolic activity was cited as the reason for increased respiration rate during the first 24 h following irradiation [12, 179]. However, the content of O_2 increased gradually on further storage. Such an observation was also reported in mushrooms subjected to gamma irradiation were linked to the reduction in metabolic activity during storage [179]. After a certain storage period (5 d for ash gourd, 5 d for drumstick and 7 d for pumpkin), no significant ($p < 0.05$) change in the O_2 content was observed, indicating attainment of equilibrium between package headspace and atmosphere. O_2 content less than 2% in

package headspace can result in anaerobic conditions leading to generation of off flavors and possible growth of pathogens like *Clostridium botulinum* [180]. In the present study, the O_2 concentrations observed for the processed samples during storage were between 16 to 19 %, thus maintaining aerobic conditions within the package. Processing and packaging conditions chosen in the present study were therefore suitable for the RTC products of the selected vegetables.

3.2.4. Aroma quality - chemical analysis of free and bound aroma compounds and identification of key odorants

Aroma is an important quality parameter that decides consumer choice and acceptability of food. It is contributed by volatile compounds that are generally hydrophobic, and usually occur in trace levels (ppm or ppb). These constituents show considerable variation in both the nature and concentration in different food stuff. The nature of the food matrix affects the concentration of volatile composition in the headspace and consequently their perception by the consumer. In fresh food, flavor results from natural compounds produced by enzymatic degradation during harvesting and processing [181]. Moreover, the flavor of cooked food is due to numerous chemical and enzymatic reactions that are influenced by temperature. Despite the presence of several volatile aroma compounds in a food matrix, not all of them are responsible for the characteristic odor of a food product. It is known that only a small portion of the large number of volatiles occurring in a food matrix contributes to its overall perceived odor [182]. Further, these molecules do not contribute equally to the aromatic profile of a sample. Sometimes, one substance alone is

able to reflect the approximate flavor of a product and, in this case, it is called “impact compound”. But, in some circumstances, it is the combination of substances that collectively interacts with the receptors from the nasal mucosa and is interpreted by the brain to create a sensory impression typical for each product [182]. In general, the sensory importance of an odor-active compound depends on its concentration in the matrix, and on the limit of detection by human nose. Therefore, there is a great interest to determine the contribution of various constituents in aroma isolate towards the overall flavor of a product.

Intensive research carried out over the past three decades has demonstrated that, besides the volatile constituents that contribute to odor, several aroma compounds accumulate as non-volatile and flavorless glyco-conjugates, which make up a reserve of aroma to be exploited [183]. These glyco-conjugates are shown to contribute finer notes to food. Although odorless, they are able to release free aroma compounds by enzymatic or chemical hydrolysis during processing and storage [39]. In the past few years, analysis of flavor precursors in fruits, vegetables and spices have received increased attention. These precursors in many cases have been shown to be more abundant than the free form to the extent of 70-90 %. However, very few reports exist on the nature of the glycosidic precursors in the vegetables.

Despite the fact that vegetables impart characteristic flavor (aroma, taste and color) to the cuisine, very few reports exist on the nature of their aroma compounds. In the green form, aroma of majority of the vegetables is indistinguishable from each other. Cooking results in liberation of their characteristic aroma from their precursors. The nature of these

precursors has been exhaustively investigated in the vegetables of the Cruciferae family, where in liberation of aroma from glucosinolates has been reported. No report, however, exists on the nature of these precursors in majority of the vegetables, specifically of the Indian origin. To the best of our knowledge, only one report exists on the nature of free aroma compounds in ash gourd and pumpkin, while the drumstick aroma has not been explored so far [106, 184]. The glycosidic aroma precursors in these vegetables have not been studied till date. No study exists on the aroma impact compounds that contribute to the characteristic odor of these vegetables. It was therefore of interest to investigate free and bound aroma compounds of these vegetables and characterize the key odorants.

3.2.4.1. Ash gourd

3.2.4.1.1. Free aroma analysis: The free aroma of ash gourd was extracted employing three different techniques – simultaneous distillation extraction (SDE), high vacuum distillation (HVD) and solid phase micro extraction (SPME). The detailed methodology adopted has been described in section 2.2.2.1.1. Although a widely used technique, SDE has the disadvantages that some of the heat labile constituents may undergo degradation, or the low boiling volatiles may be lost at the temperatures employed [185]. A characteristic cooked odour is also perceived in many cases. Milder techniques such as high vacuum distillation (HVD) employing distillation under reduced pressure and SPME have therefore been attempted to obtain isolate that represent truer aroma [130]. The aroma volatiles were thus extracted using SDE, HVD and SPME in order to ascertain generation of artefacts as gas chromatographic (GC) patterns are largely influenced by extraction procedures [186]. The GC/MS chromatograms obtained for SDE, HVD and

SPME are presented in Figure 26. Table 27 provides a quantitative distribution of the constituents identified in the essential oils isolated by SDE, HVD as well as by SPME.

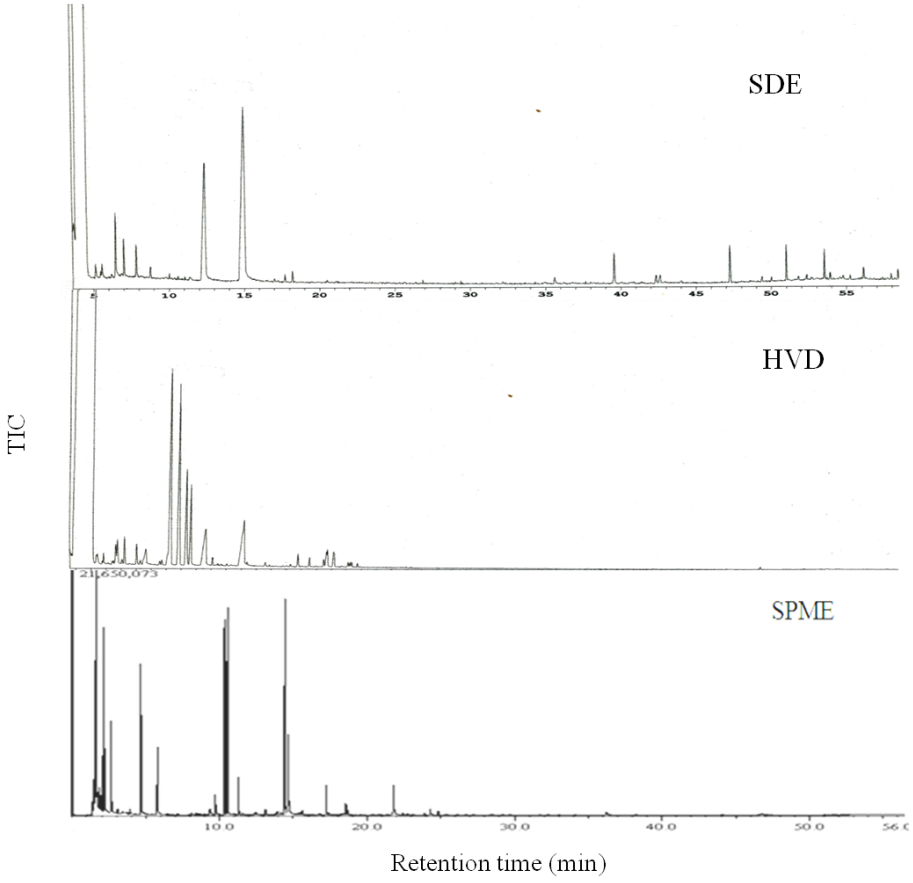


Figure 26: GC/MS profiles of volatile aroma of ash gourd obtained using SDE, HVD and SPME methods.

Table 27. Quantitative distribution (µg/kg of ash gourd) of volatile oil components identified in isolates obtained from SDE, HVD and SPME.

Compound	KI	HVD (µg/kg)	SDE (µg/kg)	SPME (µg/kg)
Alcohols				
1-Butanol	655			1.46 ± 0.15

2-Pentanol	701			0.41 ± 0.05
3-Methyl-1-butanol	735			1.29 ± 0.22
2-Methyl-1-butanol	742			0.31 ± 0.04
2,3-Butandiol	765			0.73 ± 0.19
2,4-Dimethyl-3-pentanol	825			0.1 ± 0.03
1-Hexanol	868		0.098 ±0.04	0.51 ± 0.15
1-Heptanol	965		0.012 ± 0.003	1.72 ± 0.02
2-Ethylhexanol	1021		0.068 ± 0.008	1.25 ± 0.43
Nonanol	1170		0.005 ± 0.003	0.33 ± 0.02
2-Decyn-1-ol	1275			0.15 ± 0.03
Aldehydes and ketones				
3-Methylbutanal	640			1.08 ± 1.06
2-Pentanone	675			1.09 ± 0.06
2-Ethylbutanal	742			0.43 ± 0.17
4-Methyl-3-penten-2-one	795			1.03 ± 0.37
Acetoin	812	7.59 ± 1.15	7.62 ± 1.18	12.16 ± 1.72
Hexanal	815			16.42 ± 2.28
2E-Hexenal	854		0.006 ± 0.001	0.37 ± 0.14
Heptanal	902			0.11 ± 0.04
6-Methyl-5-hepten-2-one	985			0.47 ± 0.17
2-Octanone	988		0.011 ± 0.009	0.61 ± 0.19
Octanal	994		0.006 ± 0.003	0.71 ± 0.08

Nonanal	1093		0.024 ± 0.006	1.38 ± 0.16
Decanal	1209		0.003 ± 0.001	0.11 ± 0.03
Terpenes				
β.-Pinene	970			0.35 ± 0.01
Sabinene	976			0.51 ± 0.09
Indene	1025			0.36 ± 0.04
l-Limonene	1032			0.47 ± 0.17
α-Terpineol	1185		0.003 ± 0.001	0.21 ± 0.04
Nerolidol	1550		0.014 ± 0.009	
Others				
Methyl Butyrate	735			0.3 ± 0.07
Ethyl acetate	806	0.014 ± 0.006	0.039 ± 0.006	53.94 ± 5.46
n-Butyl acetate	815			0.36 ± 0.11
3-Methyl butyl acetate	875			0.4 ± 0.06
Methyl hexanoate	915			0.22 ± 0.06
3E-Hexenoic acid	1010			0.2 ± 0.04
Verbenyl acetate	1255			0.09 ± 0.01

Data are expressed as mean ± standard deviation (n = 3).

Irrespective of the extraction techniques used, acetoin was found to be the major constituent identified. It is proposed to originate from a side reaction during the biogenesis of valine and leucine [187]. Ethyl acetate was the only other constituent detected in all the three extracts. Unlike SDE and SPME, however, the GLC profile of

HVD isolates was characterized by a negligible content of aliphatic C6-C10 alcohols and aldehydes such as hexanol, heptanol, 2-ethylhexanol, hexanal, heptanal, and octanal. The C5, C6, and C8 alcohols and carbonyl compounds, responsible for green odour of several vegetables, are known to be derived from linoleic and linolenic acids [188].

The GC/MS profile of SPME sample was characterized by both, a higher number of compounds and a significantly higher content of individual constituents as compared to that obtained from SDE and HVD. A total of 37 volatile compounds were detected in the headspace-solid phase microextraction (HS-SPME) of ash gourd. Alcohols (10), carbonyls (13), terpenes (6) and fatty acid esters (7) were the principal chemical classes identified in HS-SPME. Major compounds detected in SPME, that were not present in the other two extraction techniques were: 1-butanol (1.46 µg/kg), 2,3-butanediol (0.73 µg/kg), 3-methyl-1-butanol (1.29 µg/kg), 4-methyl-4-penten-2-one (1.03 µg/kg), 3-methylbutanal (1.08 µg/kg), hexanal (16.42 µg/kg), 6-methyl-5-hepten-2-one (0.47 µg/kg), sabinene (0.51 µg/kg), limonene (0.47 µg/kg) and indene (0.36 µg/kg). The volatile profile of ash gourd comprises a large proportion of esters of aliphatic acids (Table 27). Use of SPME resulted in the detection of a large number of low molecular weight esters, such as methyl butyrate (0.3 µg/kg), n-butyl acetate (0.36 µg/kg), 3-methyl butyl acetate (0.4 µg/kg), methyl hexanoate (0.22 µg/kg) and trans-verbenyl acetate (0.09 µg/kg). These observations indicated that SPME was more efficient for the extraction of volatile aroma compounds from ash gourd. Higher extraction of alcohols and esters by SPME in comparison to SDE has been previously reported in volatile profile of beans

[181]. The evaporation step during SDE may increase the loss of the most volatile compounds [181].

In an earlier study conducted by Wu et al. on volatile compounds of ash gourd, they identified E-2-hexenal (green fruity, pungent vegetable like odour), n-hexanal (fatty green grassy) and 3-methyl-1-butanol (fruity, alcoholic) as the major volatile constituents of this vegetable [106]. These compounds were also found as major aroma volatiles in the present study. However, other volatile constituents such as n-hexyl formate, 1-octen-3-ol, undecen-1-ol, 2-hexyl furan, deca-2, 4-dienal, isoamyl alcohol and 2-heptenal earlier reported by Wu et al. were not detected by us in this study [106]. It is interesting to note that despite a high content of acetoin detected in the essential oil in our study this compound was not earlier reported even in trace amounts as a constituent of the ash gourd volatiles. The variation observed in aroma composition could be due to the difference in variety and geographical origin of the vegetable studied.

3.2.4.1.2. Bound aroma analysis

The bound aroma glycosides were extracted using two different methods – XAD and solid phase extraction (SPE) using C18 cartridges (section 2.2.2.2.2.). The use of XAD as well as SPE is widely reported in literature [189]. The extracts obtained from XAD and SPE were separately subjected to acid and enzymatic hydrolysis. The released free aglycones were then subjected to GC/MS analysis. A higher number of peaks were detected in the GC profile of enzymatically hydrolyzed glycosidic isolate. This could possibly be due to mild conditions during enzymatic hydrolysis unlike the harsh conditions that prevail during acid hydrolysis resulting in significant modification of the

released aglycones [133]. This is in accordance with the earlier reports on bound aroma precursors in wine wherein enzymatic hydrolysis are known to represent the true aroma profile (without further chemical transformations) [190]. Enzymatic hydrolysis as a method for release of free aglycones from glycosidically bound aroma precursors was thus adapted for further studies.

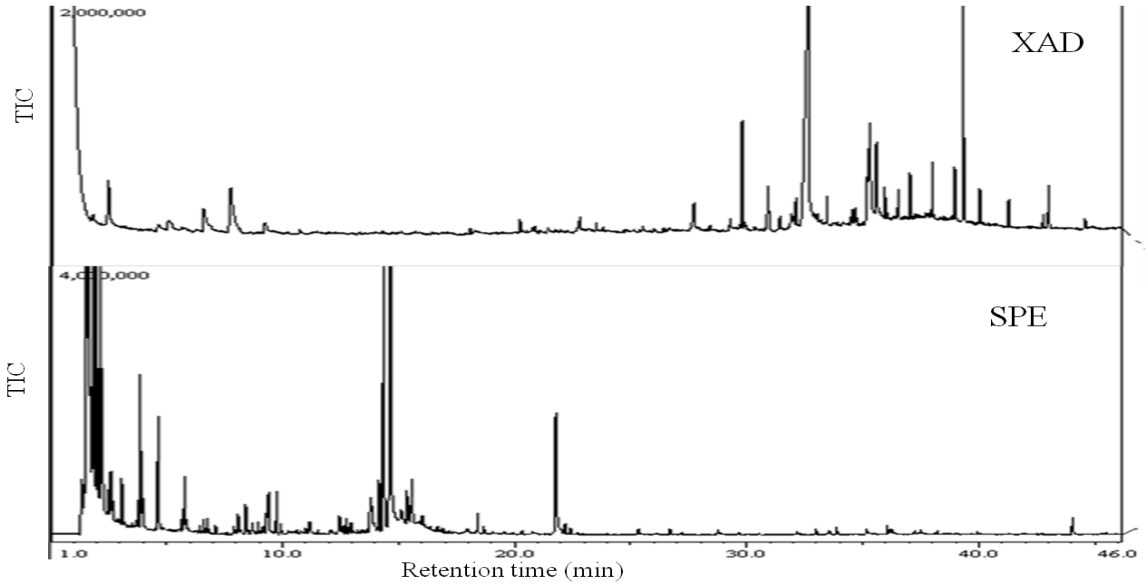


Figure 27. GC/MS profile of free aglycones released from bound aroma precursors of ash gourd obtained from XAD and SPE.

The representative GC/MS chromatograms of the released aglycones obtained after enzymatic hydrolysis of XAD and SPE isolates are shown in Figure 27. As evident from the chromatograms, significantly higher number of peaks was observed in the SPE extract. Table 28 provides a quantitative distribution of aglycones liberated from glycosidic precursors extracted by XAD and SPE. Acetoin was detected as the major aglycone released after hydrolysis of bound aroma glyco-conjugates isolated by both the methods. Acetoin accounted for 98% of the bound aroma compounds in XAD extract and 46% in the SPME

isolate. The occurrence of acetoin as a major glyco-conjugate further confirms this compound as an important aroma constituent of ash gourd. Among the other bound aroma constituents identified in XAD extract, 2-pentanol (minty), 2-ethyl-1-hexanol (floral), 3-methyl butanol (ripe onion) and vanillin (vanilla like) with low odor thresholds may also contribute to the overall odor of the vegetable.

Table 28. Quantitative distribution (µg/kg ash gourd) of free volatile components obtained from glycosidic precursors extracted by XAD and SPE.

Compound	KI	XAD (µg/kg)	SPE (µg/kg)
Alcohols			
1-Butanol	655		6.75 ± 0.26
3-Methyl-1-butanol	735	0.17 ± 0.03	23.48 ± 1.58
2-Methyl-1-butanol	742		12.31 ± 0.05
2,3-Butandiol	765		5.86 ± 0.05
1,3-Butanediol	784	0.08 ± 0.02	5.89 ± 0.05
1-Hexanol	868		3.09 ± 0.03
2,4-Hexadien-1-ol	875		0.79 ± 0.02
2-Pentanol	959	0.09 ± 0.02	2.24 ± 0.06
1-Heptanol	965		1.51 ± 0.07
2-Ethylhexanol	1021	1.68 ± 0.03	10.65 ± 0.92
4-Penten-1-ol	1072	Tr	
1-Nonanol	1171		1.17 ± 0.17
Trans-2-undecen-1-ol	1368	0.17 ± 0.03	

Dodecanol	1473	0.16 ± 0.01	
Hexadecanol	1879	Tr	
Octadeca-9,12-dien-1-ol	2035	0.17 ± 0.012	
Cis-9-octadecen-1-ol	2082	0.93 ± 0.12	
Aldehydes and ketones			
3-Methyl-butanal	640		0.14 ± 0.04
2-Pentanone	675		3.23 ± 0.24
2-Ethylbutanal	742		3.67 ± 0.1
4-Methyl-3-penten-2-one	795		24.99 ± 2.24
Acetoin	812	826 ± 3.456	138.64 ± 10.02
Hexanal	815		12.74 ± 1.18
4-Hydroxy-2-butanone	820	Tr	
2-Hexenal	854		2.19 ± 0.13
Heptanal	902		2.66 ± 0.16
Benzaldehyde	961	Tr	
6-Methyl-5-hepten-2-one	985		5.63 ± 0.18
2-Octanone	988		4.89 ± 0.77
Octanal	994		1.34 ± 0.35
Nonanal	1093	Tr	2.36 ± 0.41
Decanal	1193	Tr	0.67 ± 0.25
3-Hydroxy-4-methoxy benzaldehyde	1391	1.01 0.581	

Dodecanal	1407	0.505 ± 0.062
Terpenes		
Myrcene	955	0.8 ± 0.59
β-Pinene	970	2.28 ± 0.49
Sabinene	976	2.34 ± 0.1
Indene	1025	2.98 ± 0.15
l-Limonene	1032	5.8 ± 0.11
Cymene	1113	0.69 ± 0.18
Allo-ocimene	1125	1.11 ± 0.09
α-Terpineol	1185	1.82 ± 0.08
Others		
Methyl butyrate	735	1.45 ± 0.05
n-Butyl acetate	815	2.52 ± 0.17
Isobutyl propionate	860	2.28 ± 0.2
3-Methyl butyl acetate	875	2.34 ± 0.1
Methyl hexanoate	920	2.29 ± 0.16
Nonanoic acid	1278	1.61 ± 0.04
Methyl decanoate	1325	1.5 ± 0.08
Decanoic acid	1372	1.59 ± 0.06
Dodecanoic acid	1565	1.86 ± 0.07
Ethyl palmitate	1990	1.69 ± 0.03

Data are expressed as mean ± standard deviation (n = 3); Tr – Detected in traces

The XAD extract of ash gourd was characterized by the presence of 10 alcohols and 7 carbonyl compounds (5 aldehydes and 2 ketones). The major alcohols identified were 3-methyl-1-butanol (0.17 µg/kg), 2-ethyl hexanol (1.68 µg/kg), dodecanol (0.16 µg/kg), trans-2-undecen-1-ol (0.17 µg/kg), octadeca-9,12-dien-1-ol (0.17 µg/kg) and cis-9-octedecen-1-ol (0.93 µg/kg). As in free aroma isolate, acetoin (826 µg/kg) was also identified as the most abundant bound precursor in the XAD isolate. The other aldehydes detected in comparatively lower amounts include 4-hydroxy-2-butanone, benzaldehyde, 3-hydroxy-4-methoxy benzaldehyde, nonanal, decanal and dodecanal.

SPE isolate on the other hand, was characterized by the presence of total 42 compounds with 11 alcohols, 13 carbonyl compounds, 8 terpenes and 10 fatty acids or esters. The major compounds identified could be classified as alcohols: 3-methyl-1-butanol (23.48 µg/kg), 2-methyl-1-butanol (12.31 µg/kg), 2-ethyl hexanol (10.65 µg/kg); carbonyl compounds: acetoin (138.64 µg/kg), 4-methyl-3-penten-2-one (25 µg/kg), hexanal (12.74 µg/kg) and terpenoids: limonene (5.8 µg/kg), β-pinene (2.28 µg/kg), sabinene (2.34 µg/kg) & indene (2.98 µg/kg). Apart from the compounds which were identified in XAD extract, alcohols such as butanol, 1-nonanol, 2, 3-butanediol, 2-methyl-1-butanol, hexanol, heptanol and carbonyl compounds including 4-methyl-3-penten-2-one, 2-pentanone, 2-hexenal, heptanal, 6-methyl-5-hepten-2-one, 2-octanone were the other major compounds identified in the SPE extract. Also certain terpenes such as β-pinene, sabinene, myrcene, cymene, limonene, indene and α-terpineol were identified only in the SPE extract. The presence of glycosidic precursors of terpenes has been widely reported in literature [191]. Some higher aldehydes and alcohols such as dodecanal, dodecanol,

hexadecanol, octadeca-9,12-dien-1-ol, cis-9-octadecen-1-ol, which were present in XAD extract were not detected in the SPE extract. However, these high molecular weight compounds with high boiling points and odor threshold values, may not contribute significantly to the overall aroma of the vegetable. The above results further confirm SPE as an efficient technique for extraction of bound aroma glycosides from ash gourd, confirming earlier reports on the suitability of SPE for extraction of aroma glycosides from food stuffs [192].

3.2.4.1.3. Identification of key odorants

Aroma active compounds play a major role in imparting characteristic odor of a food. Among the several compounds that make up the aroma isolate, a single or a very few compounds in combination are known to contribute to its characteristic odor. The presence of acetoin in considerable amount, both in free and in bound form suggests its possible role as an aroma active compound of the vegetable. The study was thus focused further on the identification of the most potent odorants of ash gourd. GC–O method based on detection frequency (Global Olfactometry Analysis) of odors was employed as the method was rapid and required no trained panelist. Eight odor active compounds were sniffed by at least more than four assessors during olfactometric analysis of volatiles from SDE and SPME that were injected separately on a DB-5 column. Table 29 presents odor descriptors perceived by the panel for the aroma constituents in order of elution in this column. A variety of odor qualities such as cut ash gourd, green, herbaceous, fatty green were perceived. The nine judges constituting the sensory panel agreed that the odour of the extract was typical buttery green vegetable like. Two regions at Rt 4.5–5.5 min and

17.5–19.0 min corresponding to the cut ash gourd and fatty green, respectively, closely resembled the odor of the vegetable.

Table 29. ODP analysis of ash gourd SDE oil

Rt (min)	Aroma perceived	KI	Compound identified	OAV
4.0-4.5	Fruity	806	Ethyl acetate	5.8
4.8 – 5.67	Buttery	812	Acetoin	9522.5
9.5-9.7	Green-fruity/vegetable like	854	2E-Hexenal	352.9
10.00	Green flowery	868	Hexanol	39.2
13.75-14.00	Earthy/ herbaceous	965	Heptanol	4000
16.85-17.15	Fruity	988	2-octanone	220
17.53-17.65	Fatty green	994	Octanal	8571
17.75-17.95	Floral	1021	2-Ethyl hexanol	ND
18.5-19.0	Green/fatty/soapy	1093	Nonanal	24000
21.35-21.54	Fatty/green	1170	Nonanol	100
21.90-22.05	Fruity/anise	1185	α-Terpineol	8.57
22.15-22.25	Green/nutty	1193	Decanal	1500
25.52-25.92	Dry wood/hay	1550	Nerolidol	ND

ND – not detected

The odor detection frequency chromatogram of the volatile constituents analysed by GC–O is shown in Figure 28. The DF data showed highest frequencies for acetoin (buttery/cut ash gourd type), octanal (fatty green), nonanal (green/fatty/soapy) while somewhat lower frequencies were found for 2-hexenal (green fruity/vegetable like), hexanol (green

flowery), heptanol (earthy/herbaceous), 2-octanone (fruity), nonanol (fatty/green) and decanal (green/nutty). The identities of the eight compounds listed were established based on their retention index, odour quality, and mass spectral data with those of standard compounds. Among the odour active regions with highest DF, acetoin with a characteristic creamy, fatty and buttery aroma, octanal with a fatty green odour and nonanal with a green, fatty and soapy odour has not been reported earlier as an aroma compound of this vegetable.

Acetoin is widely reported to occur in apples, asparagus, black currants, banana, litchi, guava, blackberry, wheat, broccoli, brussel sprouts, cantaloupe, butter and yogurt [191, 193, 194]. It has also been proposed to be a major characteristic compound of lychee aroma [191]. Alkenals and alkanals are known to contribute fatty-oily, slightly rancid odours and are reported to be present in several vegetables [135]. Thus acetoin, octanal and nonanal could be proposed as the most potent odorants of ash gourd. The latter two compounds namely octanal and nonanal possess very low thresholds and thus were well perceived during global analysis. Despite a high odour threshold, acetoin was also well perceived by the sensory panel. This could possibly be due to the high concentration of this compound in the vegetable. A high OAV value of this compound (Table 29), even higher than octanal clearly suggests that its contribution to the overall odour of the vegetable might be significant. Chemical structures and mass spectra for most potent odorants of ash gourd are represented in Figure 29.

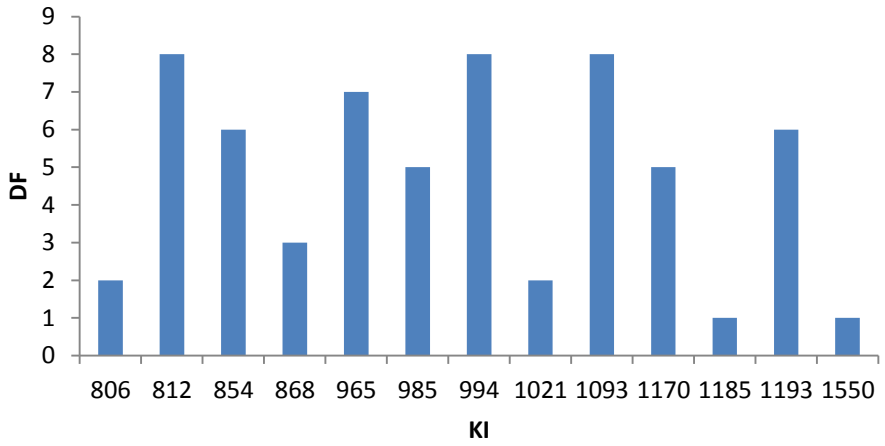


Figure 28. Odor Detection frequency chromatogram of Ash gourd SDE volatiles (Refer to Table 29 for KI)

In order to further ascertain the role of acetoin as the odour active constituent of ash gourd, the region corresponding to buttery/cut ash gourd odour, as perceived by the panel was collected from the TCD vent and further subjected to GC/MS analysis. Acetoin was the only constituent detected in the isolate (Figure 30), further confirming the role of this compound as the key aroma active compound of the vegetable.

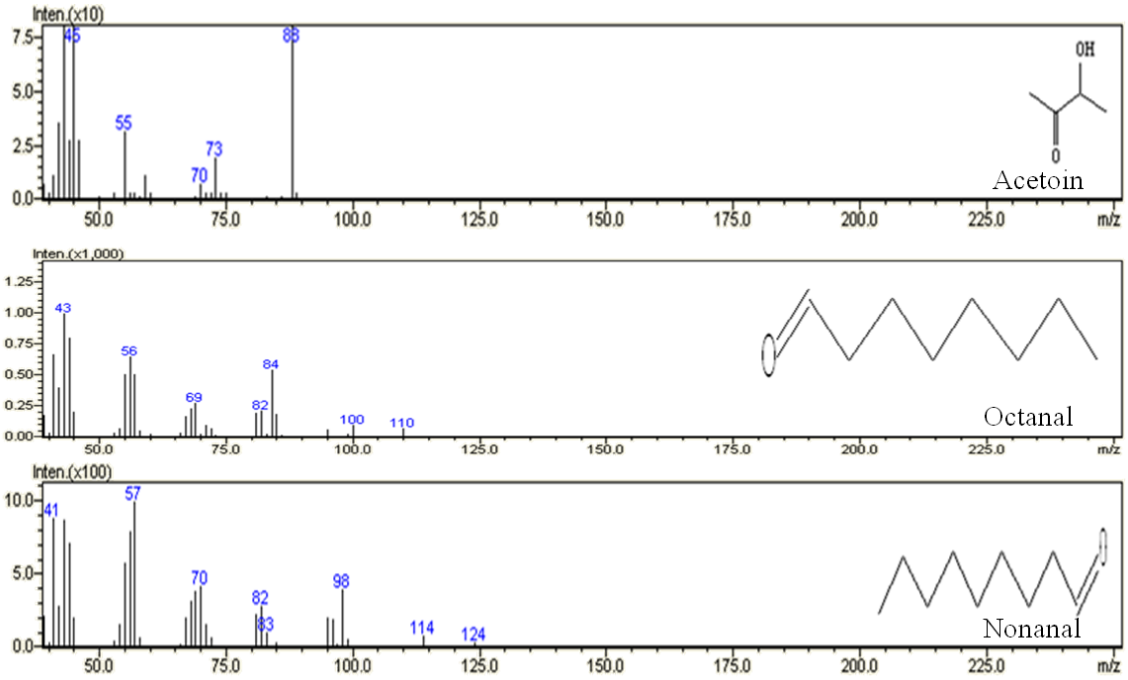


Figure 29. Chemical structures and mass spectra for most potent odorants of ash gourd

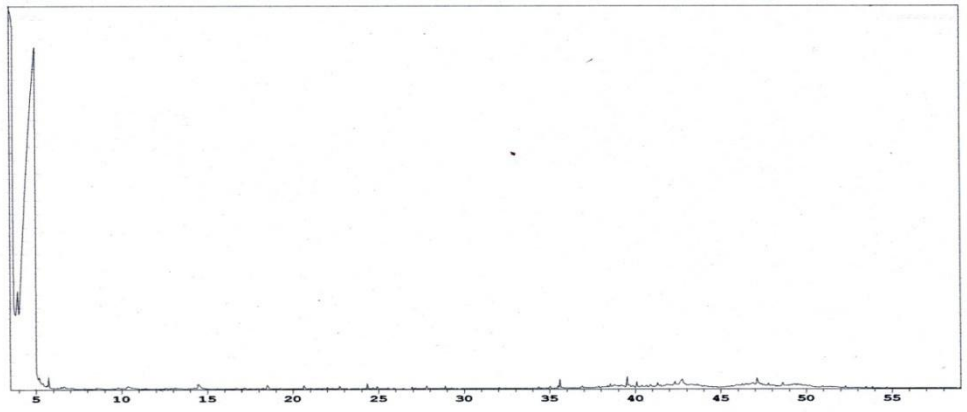


Figure 30: GC/MS chromatogram of cryotrapped fraction (4.5-5.5 min) isolated corresponding to ash gourd aroma.

3.2.4.2. Drumstick

3.2.4.2.1. Free aroma analysis

The free aroma of drumstick was extracted employing SDE and SPME (section 2.2.2.2.1). The representative GC chromatograms obtained are shown in Figure 31, while the quantitative distribution of the constituents identified is presented in Table 30.

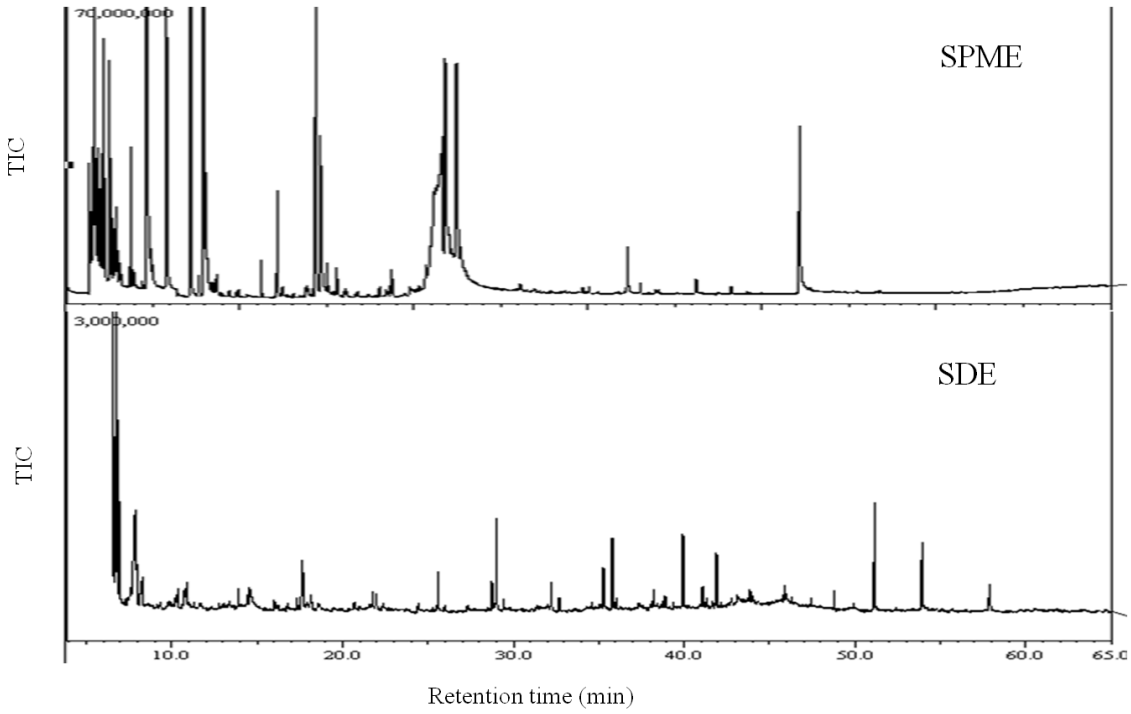


Figure 31. GC/MS profiles of volatile aroma compounds of drumstick obtained using SDE and SPME methods.

Table 30. Quantitative distribution (µg/kg of drumstick) of volatile oil components identified in SDE and SPME.

Compound	KI	SDE (µg/kg)	SPME (µg/kg)
Alcohols			
Ethanol	< 600		16.1 ± 2.08
1-PENTEN-3-OL	685		9.2 ± 0.13
1-Butanol, 3-methyl	735	2.12 ± 0.13	4.89 ± 1.48
1-Butanol, 2-methyl	742		4.41 ± 1.59
2-Penten-1-ol, (Z)	767		2.71 ± 0.29
2-Hexen-1-ol, (E)	853		3.09 ± 0.22
3-Hexen-1-ol, (Z)	857	0.016 ± 0.03	28.72 ± 3.99
1-Hexanol	868	0.02 ± 0.01	4.89 ± 0.22
1-Heptanol	965		0.63 ± 0.37
1-Octen 3 ol	985		0.18 ± 0.02
1,8-Octanediol	995	0.042 ± 0.01	
2-Ethyl hexanol	1021		7.66 ± 1.86
1-Nonanol	1171		0.55 ± 0.35
2-Decen-1-ol	1271		0.78 ± 0.19
1-Decanol	1275		8.94 ± 2.34
Aldehydes and ketones			
Acetaldehyde	< 600		12.17 ± 3.29
Iso butyraldehyde	615		14.46 ± 3.1

Butanal, 3-methyl	640		21.75 ± 1.79
1-Penten-3-one	680		10.98 ± 3.26
Pentanal (CAS)	697		5.83 ± 1.98
2-Pentenal, (E)	745		4.83 ± 0.26
3-Hydroxy-2-butanone	812	9.25 ± 0.21	21.84 ± 2.69
Hexanal	815		63.6 ± 8.38
Trans-2-hexenal	854		136.21 ± 13.53
Heptanal	899		0.88 ± 0.26
4-Heptenal, (Z)	902		0.18 ± 0.03
2-Heptenal	955		0.22 ± 0.01
Benzaldehyde	961		2.42 ± 0.14
2-Octanone	988		2.23 ± 0.52
Benzeneacetaldehyde	1043		2.34 ± 1.08
2-Octenal, (E)	1060	0.004 ± 0.001	0.56 ± 0.06
Nonanal	1093		3.44 ± 0.33
2,6-Nonadienal, (E,Z)	1154		6.08 ± 3
Decanal	1193	14.42 ± 2.15	21.05 ± 4.52
Dodecanal	1407	0.004 ± 0.001	30.99 ± 8.73
2-Decenal	1261	2.06 ± 0.05	6.28 ± 1.55
2,4-Decadienal	1315		2.2 ± 0.33
Nitrogen and sulphur containing compounds			
Propanenitrile, 2-methyl	625		12.16 ± 1.73

Butanenitrile, 2-methyl	695		3.43 ± 0.86
Propanesulfonylacetonitrile	1250		28.42 ± 8.07
2-hydroxy propane nitrile	725	8.54 ± 0.87	
Isopropyl isothiocyanate	835		109.99 ± 17.66
Dimethyl sulfone	920	0.029 ± 0.001	
Butane, 2-isothiocyanato	946		5.14 ± 0.15
Isobutyl isothiocyanate	974		14.79 ± 0.49
Benzyl nitrile	1138	0.004 ± 0.001	
Benzene isonitrile	1198		1.86 ± 0.22
2-methoxy-3-(isobutyl)-pyrazine	1204	0.005 ± 0.001	
Benzothiazole	1215	10.3 ± 1.3	19.25 ± 3.87
Terpenes and others			
Ethyl acetate	806	8.95 ± 1.65	
Z-4-Hexenyl acetate	1012	0.016 ± 0.002	
l-LIMONENE	1031		2.52 ± 1.31
Trans-Linalool oxide	1088		2.1 ± 0.12
Linalool	1098	0.004 ± 0.001	0.59 ± 0.36
α-Terpineol	1185		12.28 ± 0.24

Data are expressed as mean ± standard deviation (n = 3).

As in ash gourd, use of SPME also resulted in isolation of a higher number of aroma compounds in drumstick. The evaporation step during SDE could possibly have resulted in the loss of the most volatile compounds [181]. The GLC profile obtained from SPME

was dominated by alcohols, carbonyls and esters. A higher extractability of alcohols and esters by SPME fibre used has been reported earlier in beans by Barra et al. [181]. This could possibly explain the dominance of above class of compounds in the volatile profile.

The GLC profile obtained from SDE extract was characterized by the presence of 17 volatile compounds of which one terpene, 4 alcohols, 5 carbonyl compounds, 5 nitrogen or sulfur containing compounds and two esters were identified. The major aroma compounds present in the SDE isolate were 3-methyl-1-butanol (2.12 µg/kg), 3-hydroxy-2-butanone (9.25 µg/kg), 2-hydroxy propane nitrile (8.54 µg/kg) and ethyl acetate (8.95 µg/kg).

SPME profile, on the other hand, was characterized by the presence of 48 volatile compounds. It included 14 alcohols, 22 carbonyl compounds, 8 nitrogen and sulphur containing compounds and 4 terpenes. The major alcohol and carbonyl compounds identified were 3-hexen-1-ol (28.72 µg/kg), 2-ethyl hexanol (7.66 µg/kg), decanol (8.94 µg/kg), 3-hydroxy-2-butanone (21.84 µg/kg), 3-methyl butanal (21.75 µg/kg), 1-penten-3-one (10.98 µg/kg), trans-2-hexenal (136.21 µg/kg) and dodecanal (30.99 µg/kg).

Interestingly, the SPME profile of drumstick was characterized by the presence of various nitrogen and sulphur containing compounds. It included nitriles: 2-methyl propane nitrile (12.16 µg/kg), 2-methyl butane nitrile (3.43 µg/kg), propane sulfonyl acetonitrile (28.42 µg/kg), benzene isonitrile (1.86 µg/kg); isothiocyanates: isopropyl isothiocyanate (109.99 µg/kg), isobutyl isothiocyanate (14.79 µg/kg); and benzothiazole (19.25 µg/kg). Nitriles, isonitriles and isothiocyanates are widely reported to be present in vegetables of order Brasicales and are generated by the hydrolytic breakdown of their

precursor glucosinolates. These hydrolysis products are known to be responsible for the characteristic aroma of various vegetables of this order [58]. 2-hydroxy propane nitrile, dimethyl sufone and 2-methoxy-3-isobutyl pyrazine which were present in the SDE isolate, were not detected in the profile obtained from SPME. These could have been generated as a consequence of heat treatment employed during SDE.

Despite the fact that drumstick fruits have a distinct and pleasant aroma, no studies on the detailed aroma composition have been reported till date. The volatile aroma compounds of drumstick leaves and flowers have, however, been exhaustively explored. In a study conducted by Dev et al., effect of hot air drying on the volatiles of drumstick pods was studied using z-Nose (an electronic nose) [195]. The effect of different drying methods - microwave-assisted hot air drying (MAHD) and conventional hot air drying methods at different temperatures were investigated by these authors. Among the various volatiles detected, β-sitosterol was the major compound while 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, n-hexadecanoic acid and 9,12-octadecadienoic acid were the minor compounds (< 10 ng/g) identified by them using GC/MS. Microwave drying was shown not to cause any significant variation in the content of these volatiles. However, detailed composition of aroma volatiles was not studied by these researchers.

3.2.4.2.2. Bound aroma analysis

Figure 32 represents the GC/MS profiles of aglycones released after enzymatic hydrolysis of the bound aroma glycosides of drumstick. The quantitative distribution of the

aglycones identified in XAD and SPE hydrolyzates of drumstick are presented in Table 31.

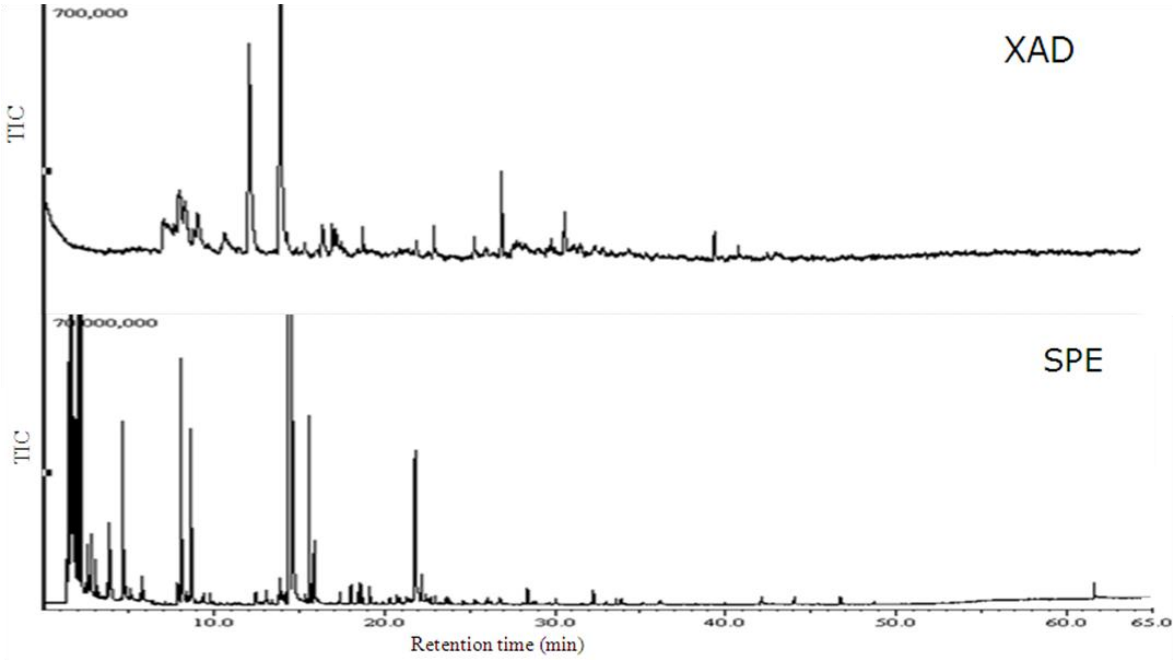


Figure 32. XAD and SPE chromatograms of drumstick

Table 31. Quantitative distribution (µg/kg drumstick) of free volatile components obtained from glycosidic precursors extracted by XAD and SPE.

Name	KI	XAD (µg/kg)	SPE (µg/kg)
Alcohols			
Ethanol	<600		10.88 ± 1.39
2-Butanol	605		36.64 ± 8.05
3-Pentanol	680		1.36 ± 1.07
2-Pentanol	690		0.18 ± 1.56
3-Penten-2-ol	710		1.88 ± 0.05

3-Buten-1-ol, 3-methyl-	725		1.02 ± 2.41
1,2-Propanediol	729		1.19 ± 2.02
1-Butanol, 3-methyl	735		12.11 ± 1.3
1-Butanol, 2-methyl	742		4.89 ± 0.14
2-Penten-1-ol, (Z)	767		0.16 ± 0.74
2-Hexanol (CAS)	780		0.77 ± 0.74
4-methyl-1-pentanol	835		0.1 ± 4.07
2-Hexen-1-ol, (E)	853		0.25 ± 6.03
3-Hexen-1-ol, (Z)	857	2.5 ± 0.97	24.35 ± 2.87
1-Hexanol	868		14.58 ± 0.04
1-Heptanol	965		1.3 ± 4.18
1-Octen-3-ol	985		0.48 ± 0.05
1-Hexanol, 2-ethyl-	1021		9.32 ± 0.03
Benzyl alcohol	1035	4.35 ± 1.07	7.34 ± 0.02
2-Octen-1-ol, (Z)	1060		0.19 ± 0.17
2-Methylphenol	1070		0.28 ± 0.13
Z-6-Nonenal	1097		1.02 ± 0.09
Benzene ethanol	1120	5.94 ± 1.05	2.06 ± 0.07
2E,6Z-Nonadien-1-ol	1158		0.5 ± 0.1
1-Nonanol	1171		1.02 ± 0.33
2-Propyl-1-heptanol	1190	3.06 ± 0.17	
1-Octyn-3-ol	1225		1.42 ± 0.14

1-Decanol	1275	1.07 ± 0.61
Aldehydes and ketones		
Acetaldehyde	<600	2.74 ± 0.24
Butanal, 3-methyl	640	1.18 ± 0.37
2-Ethylbutanal	742	0.13 ± 0.11
3-Hydroxy-2-butanone	812	14.31 ± 0.01
Hexanal	815	2.22 ± 2.46
2-Hexenal	854	2.09 ± 6.03
Heptanal	902	0.16 ± 4.07
Benzaldehyde	961	1.42 ± 0.04
Benzeneacetaldehyde	971	0.71 ± 1.98
5-Hepten-2-one, 6-methyl-	985	0.67 ± 0.31
2-Octanone	988	1.59 ± 13.12
Nonanal	1093	1.63 ± 4.18
Dodecanal	1407	0.75 ± 0.05
Nitrogen and sulphur containing compounds		
Butanenitrile, 2-methyl	695	1.65 ± 0.01
Isopropyl isothiocyanate	835	0.19 ± 0.02
1-Propene, 3-isothiocyanato-	865	0.16 ± 0.13
Terpenes and others		
1,3-Butadien-1-ol, acetate	785	0.04 ± 0.1
1-Butanol, 3-methyl-, acetate	875	0.14 ± 0.07

Ethyl pentanoate	898		0.19 ± 0.33
Myrcene	955		0.85 ± 1.09
Indene	1025		0.66 ± 0.14
Limonene	1031		0.54 ± 0.17
Trans linalool oxide	1088	11.69 ± 1.25	1.86 ± 0.1
Linalool	1098		1.19 ± 0.61
Myrcenol	1115		0.91 ± 0.12
2-Octen-1-ol acetate	1120	7.7 ± 1.17	
Thujanol	1078	2.63 ± 0.56	0.54 ± 0.17
Citronellol	1178		0.4 ± 0.09
α-Terpineol	1185		2.44 ± 0.33
trans-Geraniol	1255		0.16 ± 0.21
Thymol	1285		0.1 ± 0.12
Limonene dioxide	1290		0.87 ± 0.11
Methyl decanoate	1325		0.53 ± 0.31
Eugenol	1355		1.58 ± 6.03
Decanoic acid	1372		0.73 ± 0.02
Tetradecane	1415	0.68 ± 0.09	
Pentadecane	1500	0.81 ± 0.05	
Stearic acid	2075	1.74 ± 0.12	

Data are expressed as mean ± standard deviation (n = 3).

While only 10 aroma aglycones were detected in XAD, SPE isolate of drumstick was characterized by the presence of 62 volatile compounds. The major aglycones identified in XAD extract were 3-hexen-1-ol (2.5 µg/kg), benzene ethanol (5.94 µg/kg), benzyl alcohol (4.35 µg/kg), 2-propyl heptanol (3.06 µg/kg), trans linalool oxide (11.69 µg/kg), and 2-octen-1-ol acetate (7.7 µg/kg). Although the GC/MS profile obtained from hydrolyzed XAD extract exhibited the presence of hydrocarbons and fatty acids such as tetradecane, pentadecane, and stearic acid, they were not present in the SPE extract. However, such compounds might not affect the overall aroma perception of the vegetable. Alcohols constituted the most abundant chemical class in the SPE isolate, with 34 volatile compounds. The isolate also contained 12 carbonyl compounds including 9 aldehydes and 3 ketones. The major alcohols and carbonyl compounds identified were 3-methyl-1-butanol (12.11 µg/kg), 2-methyl-1-butanol (4.89 µg/kg), 3-hexen-1-ol (24.35 µg/kg), hexanol (14.58 µg/kg), 2-ethyl-1-hexanol (9.32 µg/kg), 3-hydroxy-2-butanone (14.31 µg/kg), 3-methyl butanal (1.18 µg/kg), 2-hexenal (2.09 µg/kg) and 2-octanone (1.59 µg/kg). Most of these volatiles were also present in free form. Apart from these, several terpenes along with fatty acids and esters were also detected in the bound aroma profile of RTC drumstick. The major terpenes identified included limonene (0.54 µg/kg), linalool oxide (1.86 µg/kg), linalool (1.19 µg/kg), α-terpineol (2.44 µg/kg), myrcene (0.85 µg/kg), limonene dioxide (0.87 µg/kg), myrcenol (0.91 µg/kg) and eugenol (1.58 µg/kg). The presence of glycosidic precursors of terpenes has been widely reported in literature [191]. However, to the best of our knowledge, no report exists on the nature of glycosidic aroma precursors of drumstick pods.

Interestingly, unlike free aroma volatiles, very few nitrogen containing compounds were detected in bound aroma profile of drumstick. As the hydrolysis was carried out with pectinase, glucosinolates might not have been hydrolyzed to release nitriles or isothiocyanates. However, the myrosinase enzyme present inherently in the vegetable might have resulted in generation of the few such compounds (2-methyl butane nitrile, isopropyl isothiocyanate and 1-propen-3-isothiocyanato) during processing.

3.2.4.2.3. Identification of key odorants

Not all volatile compounds are responsible for the characteristic odor of a food product. Consequently, there is a need to identify those that have a real olfactive impact on drumstick. GC-O employing a sensory panel was used to determine the typical odor notes of drumstick as described earlier (section 2.2.2.2.4.1). These odors were then compared with volatile compounds identified by GC/MS in order to determine key odorants of drumstick. Volatile aroma isolate was extracted by steam distillation using simultaneous distillation extraction. The process was repeated several times and the isolates obtained were pooled, concentrated and injected in GC/MS equipped with Olfactometry Detection Port (ODP). The aroma volatiles isolated from HS-SPME were also subjected to Global olfactometric analysis separately. Similar aroma notes were perceived by the sensory panel in both the extracts. Table 32 represents different aroma notes perceived by the sensory panel at different retention times. The compounds responsible for the aroma note perceived was identified using MS at corresponding retention times.

Table 32. ODP analysis of drumstick SDE oil

Rt (min)	Aroma perceived	KI	Compound identified	OAV
3.8 – 5.67	Buttery	812	Acetoin	0.03
7.75	Sulphur / rubber		ND	
8.2 – 8.7	Grassy green	815	hexanal	14.13
9.27	Sulphur / rotten	835	Isopropyl isothiocyanate	ND
9.91-10.39	Flowery – green	857	Cis-3-Hexen-1-ol	0.404
10.7-10.9	B-complex / medicinal		ND	
12.11-12.39	Boiled potato		ND	
12.71- 13.13	Boiled drumstick/ cooked vegetable	946	Butane-1-isothiocyanato	ND
13.45	Roasted peanuts		ND	
14.02-15.3	Burnt rubber/rubber	974	Isobutyl iso thiocyanate	ND
18.23- 18.5	Flowery	1043	Benzene acetaldehyde	0.468
20.6-20.9	Smoky / beany	1093	Nonanal	3.44
22- 22.7	Roasted nuts	1198	Benzene isonitrile	
23.5- 24	Boiled beany / boiled seeds of drumstick	1204	2-Methoxy-3-isobutyl pyrazine	2.5
24.7- 24.9	Boiled veg / fatty	1209	Decanal	210.5
26.00-26.30	Fresh cut drumstick	1215	Benzothiazole	0.24
27.08 – 27.2	Green drumstick	1261	2- Decenal	20.93
29.42 – 29.7	Fatty green	1291	2,4-decadienal (E,E)	31.42
32 – 32.8	Fruity/ leafy		Damascenone	ND

ND: Not Detected

Various aroma notes such as buttery, sulphury, grassy green, flowery, boiled drumstick, drumstick seed, fatty/ beany, fresh-cut drumstick, fatty green were perceived by the panelists. The nine judges constituting the sensory panel agreed that the odor of the extract was typical drumstick like. A region corresponding to 26.19-29.4 closely resembled to the odor of fresh-cut drumstick pods. The odor detection frequency chromatogram of SDE volatile constituents analyzed by GC-O is shown in Figure 33.

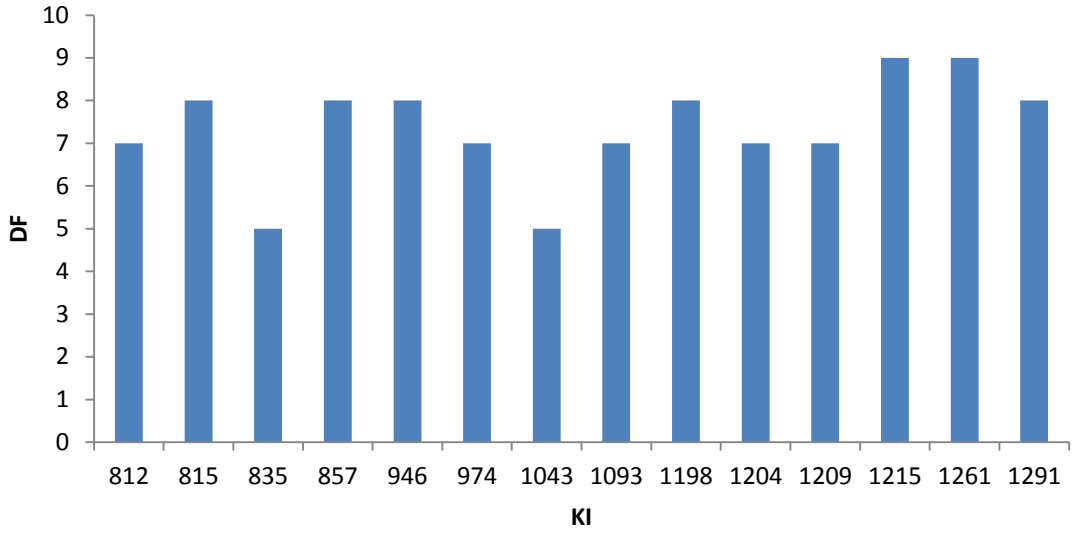


Figure 33. Odor detection frequency chromatogram of drumstick SDE volatiles (Refer table 32 for RI)

The highest detection frequency of 9 was observed for fresh-cut drumstick and green drumstick aroma. Other odor notes readily perceived by the panel were grassy green, flowery-green, boiled drumstick, roasted nuts and fatty green having a detection frequency of 8. Many other odor notes such as sulphury/ rubbery, sulphury-rotten, boiled potato, flowery, boiled beany, fruity/ leafy were perceived with comparatively lower detection frequencies (Table 32). The identities of the compounds listed were established

based on their retention indices, odor quality, and mass spectral data with those of standard compounds as listed in NIST and Wiley aroma compounds libraries. The compounds identified corresponding to the aroma notes of fresh-cut and green drumstick were decanal, benzothiazole and 2-decenal. Benzothiazole is reported to occur in various fruits and vegetables such as potato [196], asparagus, mango and cocoa. It is reported to possess sulfurous, vegetative, cooked, brown, nutty and coffee-like aroma. Alkanals and alkenals are reported to contribute green-fatty/oily odors and are reported to be present in several vegetables [135]. Unlike decanal and 2-decenal which possessed high odor activity values, the OAV of benzothiazole was quite low, however, it was readily perceived by the sensory panel.

To further ascertain the contribution of these compounds towards the characteristic aroma of the vegetable, SDE oil of drumstick was loaded on a preparative TLC with hexane: ether (80:20) as the developing solvent system. The TLC plate so developed was dried under a slow stream of nitrogen until free of solvent and then subjected to sensory analysis. The sensory panel sniffed the aromagram to locate the band corresponding to drumstick aroma. Fresh-cut drumstick odor was perceived by most of the panel members at a Rf value of 0.47. TLC plate when developed in iodine vapors resolved in six distinct bands (Figure 34). However, the band corresponding to the characteristic drumstick aroma could not be stained with iodine. Each band was separately scrapped and eluted with double distilled diethyl ether. The ether was filtered and concentrated by a slow stream of nitrogen. The concentrate so obtained was injected in GC/MS equipped with ODP for characterization of the odor. The aroma notes were then perceived by the

sensory panel at the sniffing port. Table 33 represents the Rf values of various bands observed along with the aroma perceived and major compounds identified.

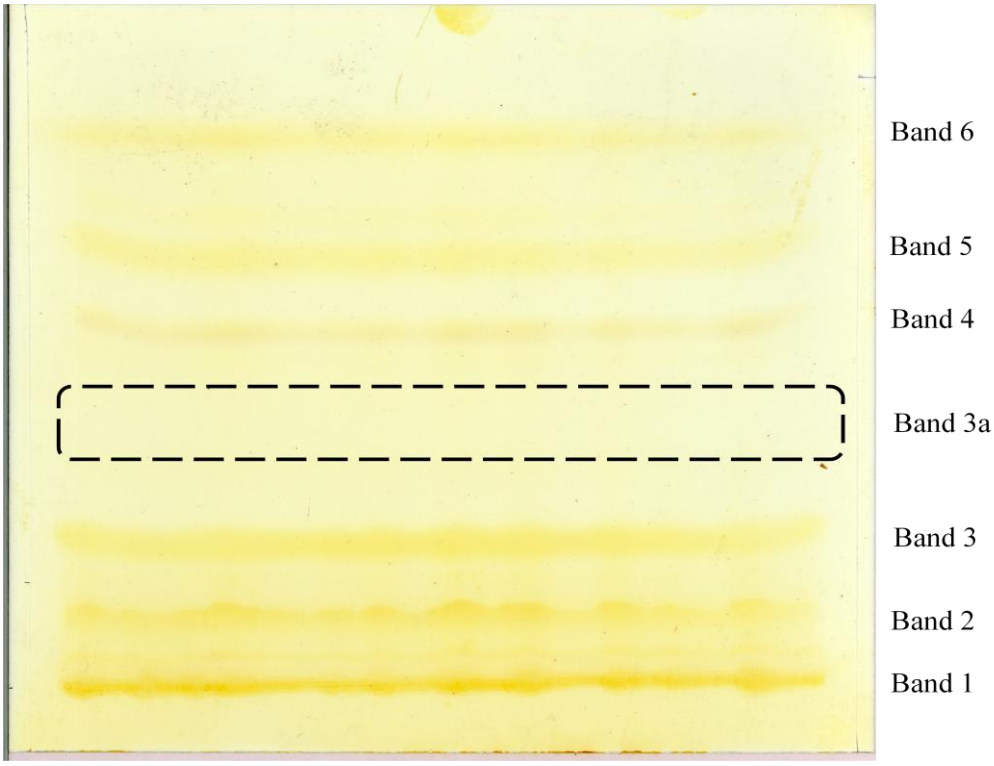


Figure 34. TLC aromagram of drumstick SDE isolate.

The GC-O analysis of the band corresponding to drumstick aroma in TLC aromagram showed the role of decanal, benzothiazole and 2-decenal as contributors to the characteristic drumstick aroma. Thus the synergistic role of decanal, benzothiazole and 2-decenal in contributing to the characteristic drumstick aroma was established. The chemical structures and mass spectra of these compounds are shown in Figure 35.

Table 33. Bands isolated from TLC aromagram of drumstick oil with their odor and major compounds identified.

Band	**Retention Factor (Aroma perceived)**	**Major Compounds identified**
Band 1 (bottom)	0 (seedy aroma)	Diacetyl sulphide, S-(+)-1,2-Propanediol, acetic anhydride, palmitic acid
Band 2	0.125 (fruity odor)	3-Hexen-1-ol, 1-Hexanol, Trans linalool oxide, alpha-Terpineol
Band 3	0.26 (Fruity / flowery)	Linalool, alpha- terpinolene
Band 3a (region between band 3 and 4; not visible in iodine)	0.47 (fresh cut drumstick aroma)	Decanal, Benzothiazole, 2- decenal,
Band 4	0.59 (Mild drumstick note + sweet smell)	L-Linalool, nonanal, decanal, E-2-Tetradecen-1-ol, Sulfurous acid, hexyl octyl ester, Cis-9-Octadecen-1-ol
Band 5	0.72 (no odor)	Sulfurous acid, 2-ethylhexyl hexyl ester
Band 6 (top)	0.91 (sulphurous smell)	No compounds could be detected

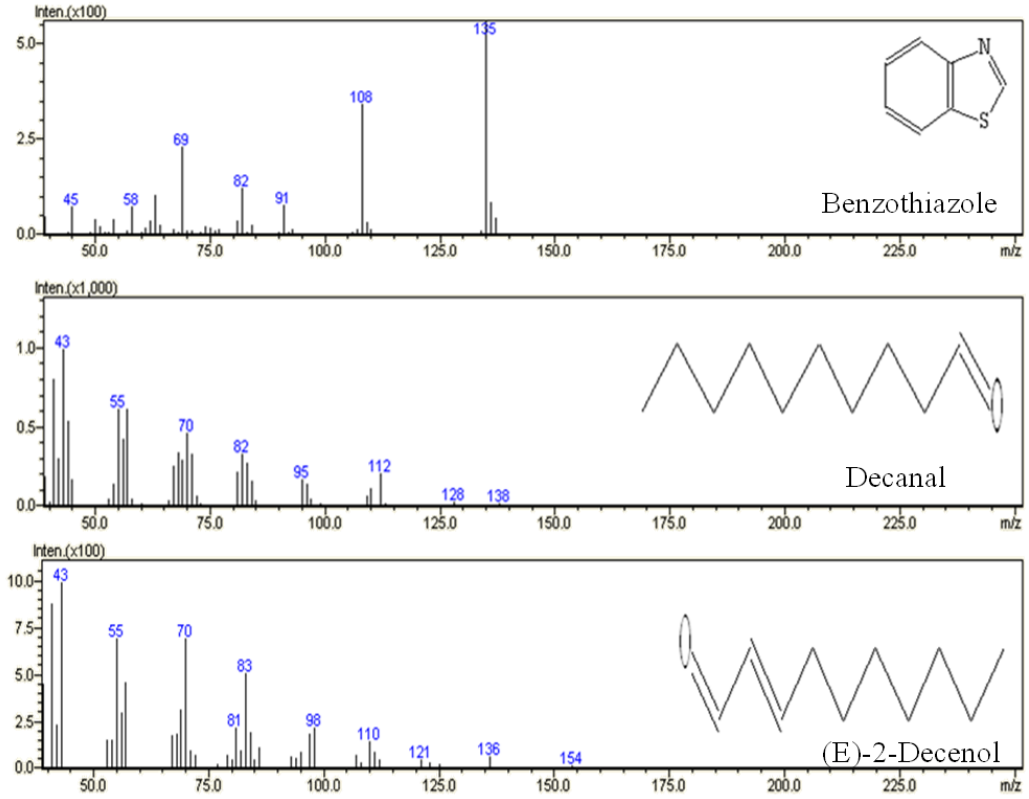


Figure 35. Chemical structures and mass spectra of most potent odorants of drumstick

3.2.4.3. Pumpkin

3.2.4.3.1. Free aroma analysis

The GC/MS chromatograms of free aroma of pumpkin as obtained by SDE and SPME are presented in Figure 36. Table 34 provides a quantitative distribution of the constituents identified in the isolates obtained from both the techniques.

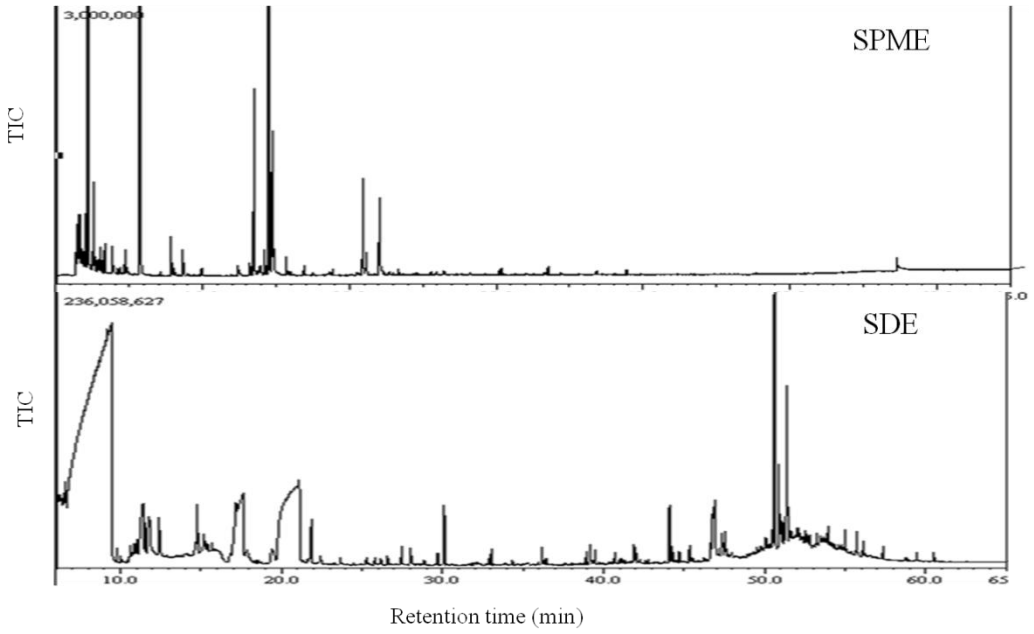


Figure 36. GC/MS profiles of SDE and SPME isolates of pumpkin

Table 34. Quantitative distribution (µg/kg of pumpkin) of volatile oil components identified in SDE and SPME

Name	KI	SDE	SPME
Alcohols			
Ethanol	<600		3.76 ± 0.08
1-penten-3-ol	685		0.84 ± 0.29
3-Methyl-1-butanol	735		1.67 ± 0.19
1-Butanol, 2-methyl-	742		0.61 ± 0.29
1-Pentanol	765		2.37 ± 0.06
Z-3-Hexenol	857	0.095 ± 0.02	0.88 ± 0.04
1-Hexanol	867		2.55 ± 0.29

(5Z)-Octa-1,5-dien-3-ol	983		1.34 ± 0.01
1-Octen-3-ol	985		19.29 ± 1.39
2 ethyl hexanol	1021		2.12 ± 0.03
benzyl alcohol	1035		0.53 ± 0.04
3-Octanol	1065		3.24 ± 0.25
2E,6Z-nonadien-1-ol	1159	1.23 ± 0.09	9.45 ± 1.65
2-Butyl-1-octanol	1250		0.19 ± 0.04
Dodecanol	1473	0.014 ± 0.05	
1-Hexadecanol	1855	0.22 ± 0.12	
Aldehydes and ketones			
Acetaldehyde	<600		1.75 ± 0.34
2-Butenal	625		1.01 ± 0.39
3-Methylbutanal	640		3.52 ± 0.12
1-Penten-3-one	680		0.69 ± 0.37
Pentanal	697		2.49 ± 0.49
(E)-3-Penten-2-one	725		0.15 ± 0.02
trans-2-Pentenal	745		0.59 ± 0.04
2-Methyl-3-pentanone	750		0.54 ± 0.01
Butanal, 2-ethyl-3-methyl-	781		0.36 ± 0.09
4-Methyl-3-penten-2-one	795		2.15 ± 0.77
Hexanal	815		44.84 ± 5.62
2E-Hexenal	854		3.46 ± 0.76

Heptanal	899		0.71 ± 0.07
2,4-hexadienal	905		0.92 ± 0.14
(Z)-2-Heptenal	955		0.86 ± 0.02
Benzaldehyde	961		0.52 ± 0.13
3-octanone	981		0.41 ± 0.28
6-methyl-5-hepten-2-one	985		0.05 ± 0.01
2-Octanone	988		0.63 ± 0.24
2E,4E-heptadienal	995		1.78 ± 0.26
Acetophenone	1059	3.28 ± 0.58	
(E)-2-Octenal	1060		1.26 ± 0.12
(Z)-6-Nonenal	1097	0.92 ± 0.14	2.48 ± 0.07
2E,4Z nonadienal	1154		0.68 ± 0.14
2E, 4E-nona-dienal	1213	0.106 ± 0.02	0.71 ± 0.01
Nonanal	1093		0.83 ± 0.03
Octadecanal	1357		0.13 ± 0.08
Dodecanal	1407		0.22 ± 0.01
Terpenes			
Cis-limonene oxide	1085		0.09 ± 0.01
Linalool	1098	0.01 ± 0.004	0.2 ± 0.05
thujone	1117		0.36 ± 0.09
3-Caren-10-al	1125	0.009 ± 0.002	
α-terpineol	1185		0.26 ± 0.04

Geranial	1197		0.39 ± 0.08
Trans-geraniol	1255	0.006 ± 0.001	
β-ionone	1455	0.007 ± 0.002	
Nerolidol	1550	0.019 ± 0.005	
Others			
n-Butyl acetate	815		0.07 ± 0.01
2-Methyl pyridine	817	0.05 ± 0.01	
Dimethyl sulfoxide	825	1.10 ± 0.05	
1-Butanol, 3-methyl-, acetate	875		0.16 ± 0.05
Dimethyl sulfone	920	3.84 ± 0.92	
Methyl decanoate	1325	0.009 ± 0.001	
Decanoic acid	1372	0.014 ± 0.01	
Tetradecanoic acid	1768	0.078 ± 0.01	
Hexadecanoic acid	1975	2.17 ± 0.16	
9Z-Octadecenoic acid (oleic)	1949	1.025 ± 0.03	
Octadecanoic acid	2075	0.578 ± 0.05	

Data are expressed as mean ± standard deviation (n = 3).

Twenty one volatile aroma compounds were detected in the SDE isolate of pumpkin. It consisted of alcohols (4), carbonyl compounds (3), terpenes (5) and nine other compounds including nitrogen and sulphur containing compounds and fatty acids along with their ester derivatives. The major aglycones present in the SDE isolate were Z-3-

hexenol (0.095 µg/kg), dodecanol (0.014 µg/kg), 2E, 6Z-nonadien-1-ol (1.23 µg/kg), hexadecanol (0.22 µg/kg), acetophenone (3.28 µg/kg), 6Z-nonenal (0.92 µg/kg), 2E, 4E-nonadienal (0.106 µg/kg), dimethyl sulfone (3.84 µg/kg), hexadecanoic acid (2.17 µg/kg) and 9Z-octadecenoic acid (1.025 µg/kg). Terpenes were detected in trace amounts in the SDE oil.

SPME isolate on the other hand, was characterized by the presence of 48 volatile compounds. It included 14 alcohols, 27 carbonyl compounds (20 aldehydes and 7 ketones), 5 terpenes and 2 esters. Aldehydes were the most abundant aroma compounds in the SPME isolate. Hexanal (44.84 µg/kg) accounted for the highest content followed by 3-methyl butanal (3.52 µg/kg), 2E-hexenal (3.46 µg/kg) and 6Z-nonenal (2.48 µg/kg). Hexanal is known to contribute green notes in vegetables. The unsaturated C6 aldehydes and particularly hexanal, has been postulated to be derived via lipid peroxidation during processing and sampling [188]. Many of these aldehydes have a low human detection threshold and are important for the flavour of tomato, cucumber, peppers and other vegetables [197-199]. Among alcohols, major volatile compounds identified were 2-methyl-1-propanol (1.52 µg/kg), 3-methyl-1-butanol (1.67 µg/kg), pentanol (2.37 µg/kg), hexanol (2.55 µg/kg), 1-octen-3-ol (19.29 µg/kg), 2-ethyl hexanol (2.12 µg/kg), 3-octanol (3.24 µg/kg) and 2E, 6Z-nonadien-1-ol (9.45 µg/kg).

Low boiling alcohols and aldehydes such as 1-penten-3-ol, 3-methyl butanol, pentanol, 3-methyl butanal, 2-butenal, 1-penten-3-one, pentanal, and hexanal, which were present as major aroma compounds in SPME isolate, could not be detected in isolate from SDE. This could be due to the masking of the initial region of chromatogram obtained from

SDE isolate by the solvent employed. Further, the evaporation step during concentration of the SDE isolate by nitrogen might have resulted in the loss of these highly volatile aroma compounds. The SDE oil was also characterized by the presence of 2-methyl pyridine, dimethyl sulfone and dimethyl sulfoxide, which were however, not detected in the SPME isolate. Such compounds could have been derived from sugars and amino acids via various chemical reactions such as Maillard reaction during SDE as a result of heat treatment [200]. SPME gave significantly better qualitative and quantitative aroma profile compared to that obtained from SDE and had a higher content of alcohols and carbonyls. Higher extraction efficiency of SPME than SDE has been reported in previous studies by various researchers [181].

The volatiles of fresh, cooked and canned pumpkin were studied earlier by Parliment et al. [184]. Combined steam distillation/solvent extraction was employed for extracting the volatiles of cooked and canned pumpkin while for fresh pumpkin, headspace concentration was used by these workers. The individual aroma compounds were identified by GC/MS. A total of 30 compounds were identified in the volatile extracts of freshly cooked and canned pumpkins and one additional compound from the raw vegetable. The major classes of compounds identified were aliphatic alcohol and carbonyl compounds (15), furan derivatives (5), and sulphur containing compounds (3). The major compounds reported to be present in the volatiles from the freshly cooked vegetable were hexanol, (Z)-3-hexenol, 2-hexenal, hexanal, and 2,3-butanedione (diacetyl). However, virtually all of the C6 aldehydes and alcohols were lost in the canned product with the major components being 2-methyl butanal, pyridine, furfural, 2,3-butanedione, and 3-

methyl butanal [184]. Other interesting compounds identified in the freshly cooked material were 3-(methylthio)propanal and a methylformylthiophene, and in the canned product 2-methyltetrahydrofuran-3-one, 2,5-dimethylpyrazine, and dimethyltrisulfide. 2-sec-butyl-3-methoxy pyrazine were shown to be present in trace concentrations (<2.5 ng/L juice) in raw pumpkin [184]. In the freshly cooked vegetable, hexanal (4.5 ng/L), (E)-2-hexenal (17 µg/L), (Z)-3-hexenol (70 µg/L), and 2,3-butanedione (7 µg/L) probably play important roles in its flavor. However, in the canned material, 2-methylbutanal (1.3 µg/L), 3-methyl butanal (2 µg/L), and dimethyl trisulfide (10 ng/L) were reported to be the more important components. Apart from few C5-C6 aldehydes and alcohols, the aroma profile obtained in the present study was found to be different from that earlier reported. This difference might be due to different variety or geographical origin of the vegetables studied.

3.2.4.3.2. Bound aroma analysis

No report exists on the nature of bound aroma glycosides of pumpkin till date. The representative GC/MS chromatograms of the free aglycones obtained from their precursors are shown in Figure 37. The quantitative distribution of these aglycones in the two isolates is presented in Table 35.

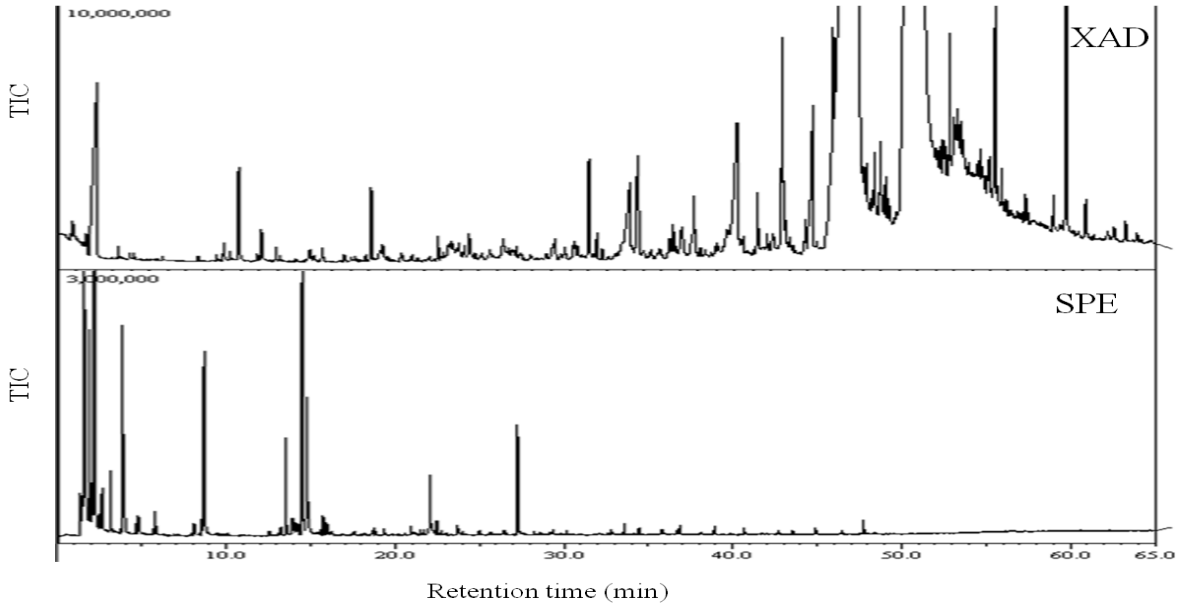


Figure 37. GC/MS profile of hydrolyzed aroma glycosides of pumpkin obtained from XAD and SPE isolates.

Table 35. Quantitative distribution (µg/kg of pumpkin) of volatile oil components identified in XAD and SPE isolates.

Name	KI	XAD	SPE
Alcohols			
1-Penten-3-ol	685		0.76 ± 0.04
3-Methyl-1-butanol	735		9.67 ± 1.66
1-Butanol, 2-methyl-	742		5.52 ± 1.02
1-Pentanol	765		1.85 ± 0.65
2-Buten-1-ol, 3-methyl-	772		0.09 ± 0.03
Z-3-Hexenol	857	0.23 ± 0.04	1.08 ± 0.31

(E)-2-Hexen-1-ol	853		1.08 ± 0.38
1-Hexanol	867	0.131 ± 0.03	13.49 ± 2.18
Heptanol	965		0.34 ± 0.07
1 Octen-3-ol	985	0.195 ± 0.06	7.03 ± 1.31
Phenol	940	0.184 ± 0.05	
3-Octanol	1065	0.558 ± 0.08	1.59 ± 0.48
2-Ethylhexanol	1021	0.519 ± 0.12	1.56 ± 0.23
Benzyl alcohol	1035	2.125 ± 0.28	1.02 ± 0.28
2-Octen-1-ol, (Z)-	1060		0.07 ± 0.01
1-Octanol	1065		0.37 ± 0.06
Phenylethyl Alcohol	1120	0.971 ± 0.04	0.64 ± 0.18
trans,cis-2,6-Nonadien-1-ol	1159	0.084 ± 0.01	0.66 ± 0.15
2,6-Dimethyl-octa-2,6-dien-1-ol	1235	0.193 ± 0.03	
2-Methoxy-4-vinylphenol	1312	1.26 ± 0.14	
Tetradecanol	1654	0.98 ± 0.19	
Hexadecanol	1855	2.17 ± 0.33	
Aldehydes and ketones			
3-Methylbutanal	640		1.33 ± 0.27
1-Penten-3-one	680		0.22 ± 0.16
Hexanal	815	1.44 ± 0.31	1.61 ± 0.53
2-Hexenal	854		0.2 ± 0.08
2-Heptanone	870		0.13 ± 0.01

Heptanal	899		0.08 ± 0.02
Benzaldehyde	961	0.11 ± 0.02	0.2 ± 0.02
6-Methyl-5-hepten-2-one	985		0.19 ± 0.03
2-Octanone	988		1.59 ± 0.25
2-Hydroxy-benzaldehyde	1035		0.15 ± 0.01
Nonanal	1093	0.064 ± 0.02	0.18 ± 0.09
6Z-Nonenal	1097	2.15 ± 0.18	0.27 ± 0.07
Dodecanal	1407	0.06 ± 0.01	0.09 ± 0.03
3-Methoxyacetophenone	1415		8.78 ± 1.53
2Z-Decenal	1259	0.744 ± 0.24	
Tetradecanal	1575	0.525 ± 0.13	
Terpenes			
Limonene	1032	0.021 ± 0.005	0.19 ± 0.09
Linalool	1098		0.41 ± 0.03
Methyl nonanoate	1225		0.25 ± 0.05
Isomenthol	1140	0.154 ± 0.06	0.68 ± 0.09
α-Terpineol	1185		1.03 ± 0.26
Cis-Limonene dioxide	1290		1.24 ± 0.6
E-Citral	1197		0.17 ± 0.03
cis-Geraniol	1255		0.33 ± 0.01
Vanillin	1412	0.894 ± 0.05	
Others			

Ethyl propionate	703		0.34 ± 0.04
Butyl acetate	815	0.095 ± 0.03	
3-Methyl-1-butanol acetate	875	0.133 ± 0.02	
Octanoic acid	1165	0.607 ± 0.09	
2,3-Dihydro benzofuran	1225	0.844 ± 0.06	
Nonanoic acid	1278	1.46 ± 0.15	
Decanoic acid	1278	1.10 ± 0.13	0.32 ± 0.04
Menthyl acetate	1304		0.41 ± 0.24
Dodecanoic acid, methyl ester	1325	0.06 ± 0.001	0.54 ± 0.2
Dodecanoic acid	1565	13.96 ± 2.05	
2-Octyl furan	1275	2.15 ± 0.24	
Tetradecanoic acid, methyl ester	1695	0.15 ± 0.002	0.37 ± 0.05
Hexadecanoic acid, methyl ester	1935	3.04 ± 0.12	
Eicosanoic acid, methyl ester	2105		0.28 ± 0.03
Octadecanoic acid	2075	3.89 ± 0.24	
9Z-Octadecenoic acid	1949	8.37 ± 1.25	

Data are expressed as mean ± standard deviation (n = 3).

A total of 36 compounds were detected in the XAD extract of pumpkin. The major chemical classes were alcohols (13), carbonyl compounds (7), terpenes (3), fatty acids and esters (11). Two furan derivatives 2,3-dihydro benzofuran and 2-octyl furan were also detected in the XAD extract. Although furan derivatives were reported earlier in pumpkin by Parliment et al. they were not detected in the free form in the present study

[184]. This might be due to low concentration of these compounds which is beyond the detectable limit of GC/MS. The major compounds identified could be classified as alcohols: benzyl alcohol (2.12 µg/kg), phenyl ethyl alcohol (0.97 µg/kg), 2-methoxy-4-vinyl phenol (1.26 µg/kg), tetradecanol (0.98 µg/kg), hexadecanol (2.17 µg/kg); aldehydes: hexanal (1.44 µg/kg), 6Z-nonenal (2.15 µg/kg); fatty acids and esters: nonanoic acid (1.46 µg/kg), decanoic acid (1.10 µg/kg), dodecanoic acid (13.96 µg/kg), methyl ester of hexadecanoic acid (3.04 µg/kg), octadecanoic acid (3.89 µg/kg), 9Z-octadecenoic acid (8.37 µg/kg); and 2-octyl furan (2.15 µg/kg).

The GC profile of the SPE isolate on the other hand, was characterized by the presence of 45 compounds with 17 alcohols, 14 carbonyl compounds, 8 terpenes and 6 fatty acids or esters. Among alcohols, the major components were 2-methyl-1-propanol (8.55 µg/kg), 3-methyl-1-butanol (9.67 µg/kg), hexanol (13.49 µg/kg), 1-octen-3-ol (7.03 µg/kg), 3-octanol (1.59 µg/kg) and 2-ethyl hexanol (1.56 µg/kg); while among carbonyl compounds, 2-methyl butanal (1.33 µg/kg), hexanal (1.61 µg/kg), 2-octanone (1.59 µg/kg) and 3-methoxy acetophenone (8.78 µg/kg) were identified as the major compounds. Major terpenes include α-terpineol (1.03 µg/kg) and cis-limonene dioxide (1.24 µg/kg) with others present in comparatively lower amounts. Very few fatty acids and esters were detected in the SPE isolate in comparison to that in the XAD extract. Most of the alcohols identified in the glycosidic fraction were also detected in the free aroma profile of the vegetable. Interestingly, many of the C5-C6 alcohols, carbonyl compounds and terpenes, which were the major components in the SPE isolate were not present in XAD isolate. Isolate from XAD was dominated by high boiling alcohols such

as tetradecanol, hexadecanol, and fatty acids and esters, which were not present in the SPE isolate. Owing to their low volatility, these compounds may not play a significant role in contributing the characteristic aroma.

3.2.4.3.3. Identification of key odorants

Many aroma compounds have been earlier reported in pumpkin. However, no sensory correlation of the identified compounds with the actual odor of the vegetable was established. It was therefore of interest to study the key odor compounds contributing towards the characteristic aroma of the vegetable. GC-O analysis was separately carried out using both SPME and SDE isolate. The headspace volatiles as extracted using SPME fibre were desorbed (270 °C) in GC/MS equipped with ODP. The isolates obtained after repeated simumtaneous distillation extractions were pooled, concentrated and also subjected to GC-O analysis. Various aroma notes were perceived by a sensory panel of 9 members at the sniffing port. Table 36 represents various aroma notes perceived at different retention times at the sniffing port along with the identities of the corresponding compounds, established using MS data and RI values as reported in the literature. Figure 38 shows a plot between detection frequency and RI of the compounds identified corresponding to the various aroma notes.

Table 36. ODP analysis of pumpkin aroma (SDE)

Rt (min)	Aroma perceived	Compound identified	KI	OAV
7.12-7.30	Grassy green	hexanal	801	10
7.5-8.30	Sweet green	ND		
10.65-10.82	Fruity-green	Heptanal	901	0.24
9.73-10.10	Fruity	Hexyl acetate	1007	0.035

11.05-11.29	Roasted peanuts/ popcorn	Vinyl acetate	1015	ND
16.00-16.2	Husk (rice husk)	2-Ethyl hexanol	1021	7.85E-05
17.34-17.5	Mustard/ sulphury	ND		
18.00-18.4	Oily green	Acetophenone	1059	0.05
21.6-21.76	Cucumber	2E, 6Z- Nonadienol	1159	9450
21.77-22.08	Pumpkin	Z-6-Nonenal	1097	124
19.5-19.7	Fatty/oily	Octadecanal	1357	ND

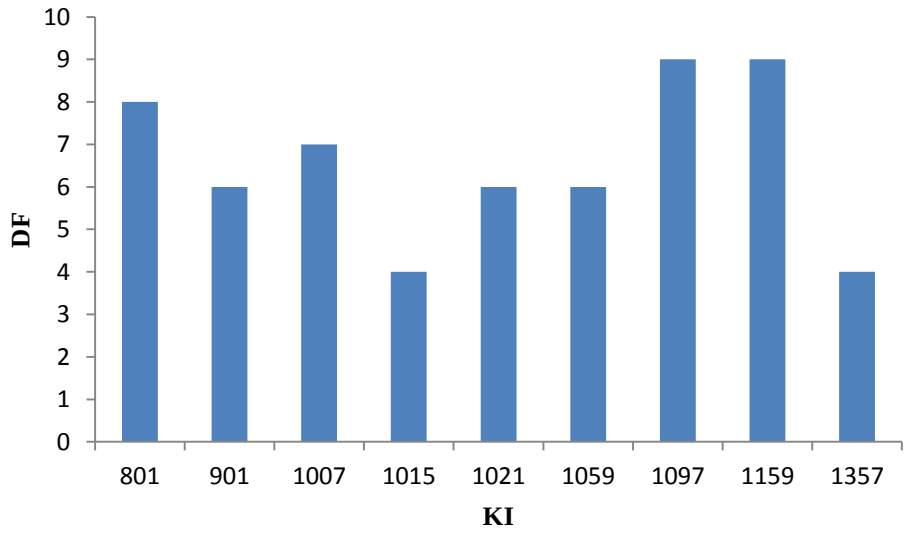


Figure 38. Odor detection frequency chromatogram of pumpkin aroma isolate (Refer Table 36 for KI)

The highest detection frequency of 9 was observed for the odor notes of fresh cucumber and pumpkin. Other aroma notes readily perceived by the sensory panel were grassy green, roasted peanuts/popcorn type aroma with a detection frequency of 8. Few other odors as perceived by the sensory panel with comparatively lower detection frequencies, included fruity-green, husk, and oily notes. The characteristic pumpkin aroma was perceived by all the panelists at a retention time 21.77-22.08 min. The corresponding

compound identified at this Rt was found to be Z-6-nonenal, previously reported as an important aroma compound of melon. The odor note corresponding to pumpkin aroma was preceded by cucucmber aroma which was found to be due to the presence of 2E, 6Z-nonadienol. The aroma of 2E, 6Z- nonadienol has been described as cucumber like while Z-6-nonenal is known to possess green cucumber and melon like with woody orris type [201].

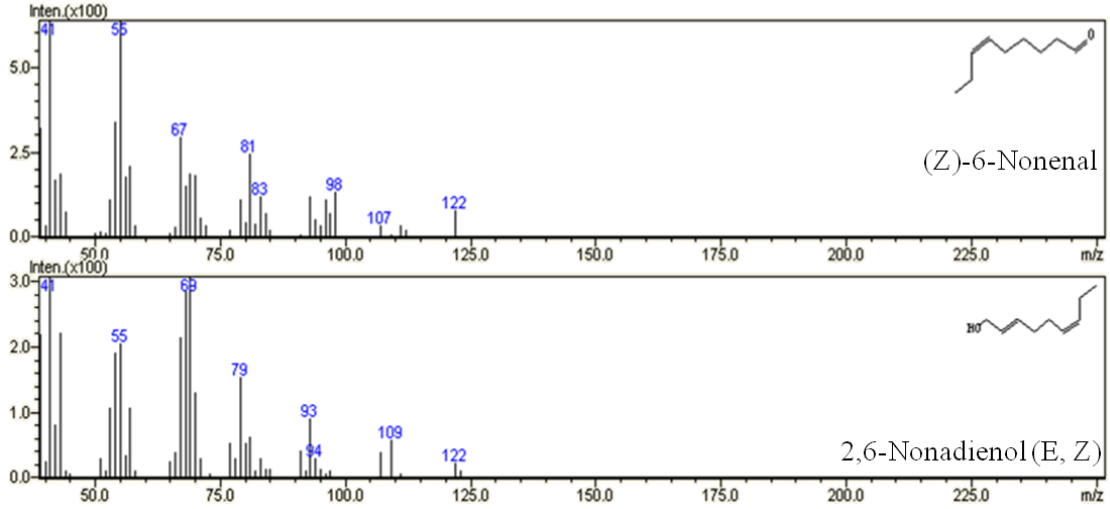


Figure 39. Chemical structures and mass spectra for most potent odorants of pumpkin

The unsaturated alcohols and aldehydes such as (E,Z)-2,6-nonadienol and Z-6-nonenal are formed during oxidation of n-3 polyunsaturated fatty acid [202, 203]. (E,Z)-2,6-nonadienol has been previously reported in muscadine grape juice with a relatively high FD factor and in musts obtained from French and Romanian Hybrids of grapes [204, 205]. Higher odor activity values (OAV) of these two compounds (Table 36) further suggested their significant contribution in the overall aroma of the vegetable. Thus, Z-6-nonenal and 2E, 6Z- nonadienol could be proposed as the most potent aroma compounds

of pumpkin. The chemical structures and mass spectra of these odorants are shown in Figure 39.

3.2.5. Effect of radiation processing on free and bound aroma profiles of the selected vegetables

Irradiation is recognized in many countries worldwide as a safe and effective method for preservation of raw and processed foods. The main benefit of irradiation includes eliminating microorganisms & insects or parasites capable of causing food spoilage and toxicity. Fresh-cut vegetables are a growing class of food stuff that has received increased interest and attention in recent years due to the convenience it provides to the consumer. One of the most important problems of storage of fresh-cut produce is the maintenance of taste and aroma that are essential parameters for their quality. Use of gamma irradiation as a potential preservation technique for improving the shelf life of fresh-cut fruits and vegetables due to the cold nature of the process holds promise.

Aroma composition plays an important role in the final quality of a food product. Radiation processing has been reported to result in significant changes in volatile profile of food products particularly those with high water content such as fresh produce [206]. Information on the impact of irradiation on the volatile composition in fresh produce is limited. Hydrolysis of aroma precursors and a resultant increase in free aroma volatiles during radiation processing has been recently reported. Radiation induced hydrolysis of aroma glycosides has been successfully demonstrated in products such as saffron, nutmeg, fenugreek and papaya [133, 207, 208, 209]. Reports on the effect of radiation

processing on these constituents in Indian vegetables are scanty. To the best of our knowledge no report exists on the effect of radiation processing on the free and glycosidically bound aroma constituents of the currently selected vegetables. It was therefore of interest to study the influence of radiation processing and storage on the volatile aroma compounds and their precursors in the selected vegetables.

As evident from the GC/MS data obtained for all the three vegetables, SPME and SPE techniques of extraction for free and bound aroma respectively were found to be better as higher number of compounds were detected and the profiles were free of interference from hydrocarbons or fatty acids as was the case with the other techniques employed. Therefore the aroma profiles obtained by SPME (for free aroma) and SPE (for bound aroma) for control and radiation processed samples at different storage durations were further examined. The free and bound aroma compounds of ash gourd, drumstick and pumpkin are depicted in tables 24-28 and 30-31. Apart from the compounds listed in the tables, certain minor peaks, which are not identified here, might also undergo changes during radiation treatment or storage. Therefore, data points at sufficiently small intervals (every 0.17 min) in each chromatogram were taken into consideration while evaluating the effect of processing steps. This however resulted in innumerable points which are difficult to analyze manually. Therefore a data reduction technique, such as principal component analysis (PCA) was employed, that can reduce the vast data points into a fewer number. This aided in explaining the inherent variation of original data. It is an unsupervised technique which is used for dimensionality reduction of multivariate data sets and allows visualization of complicated data for easy interpretation [210].

The GC/MS data obtained for free and bound aroma was therefore separately subjected to principal component analysis (PCA) for each vegetable. To know the nature of the constituents responsible for the differences among control and radiation processed samples at different days, factor loading data obtained from PCA analysis for free as well as bound aroma was analyzed separately for each vegetable.

3.2.5.1. Ash gourd

Principal component (PC) score plots obtained for free and bound aroma compounds of ash gourd are represented in Figure 40 and 41, respectively. In case of free aroma, first two principal components (F1 and F2) cumulatively explained 82.79 % of data variation while in case of bound aroma, 87.74 % variation was accounted by F1and F2.

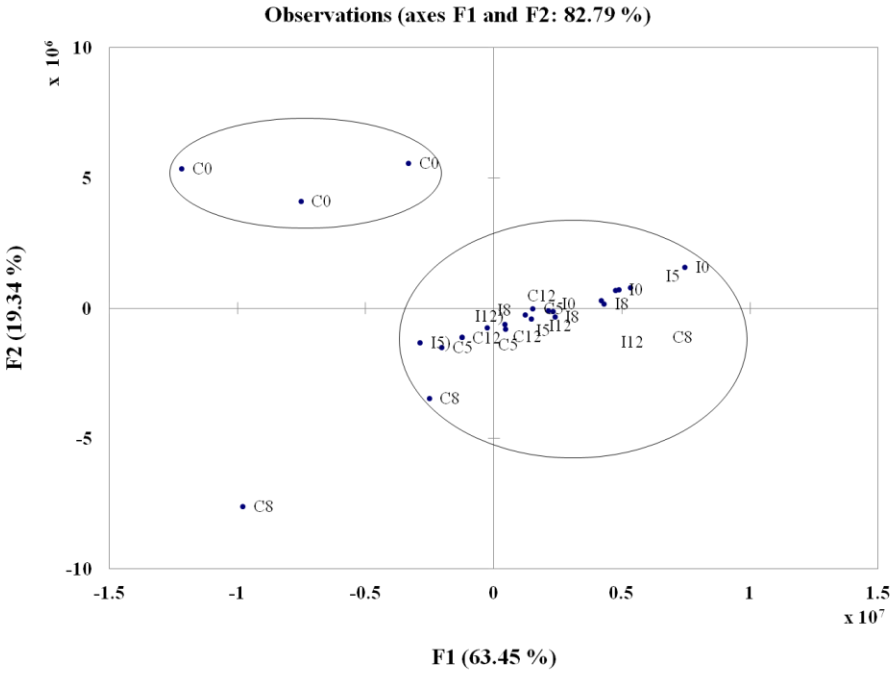


Figure 40. PCA of free aroma of ash gourd (C0, C5, C8, C12 – control samples of day 0, 5, 8 and 12; I0, I5, I8, I12 – irradiated (2 kGy) samples of day 0, 5, 8 and 12).

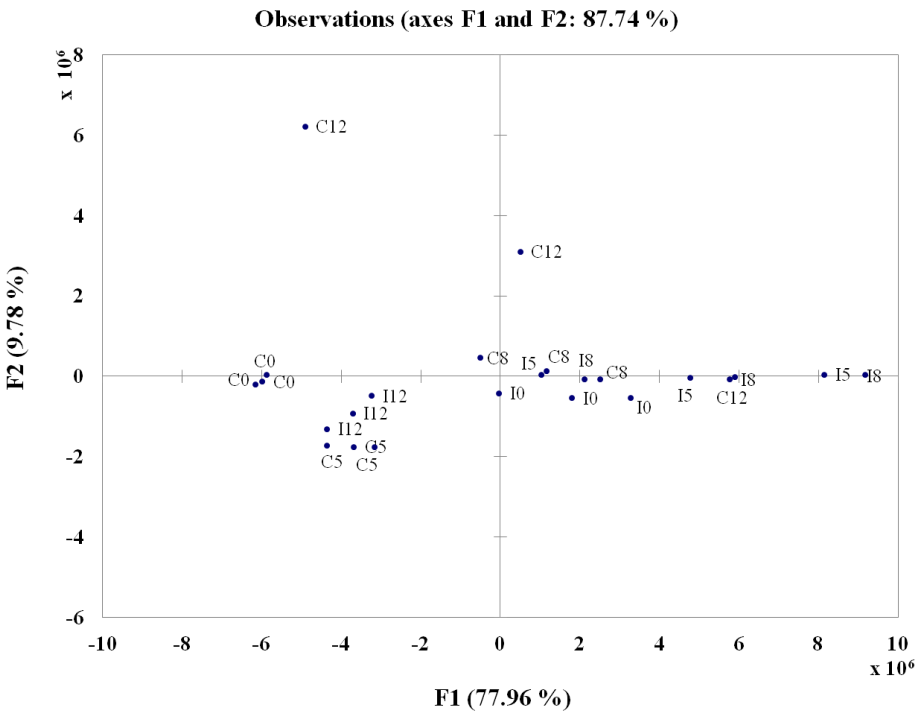


Figure 41. PCA of bound aroma of ash gourd (C0, C5, C8, C12 – control samples of day 0, 5, 8 and 12; I0, I5, I8, I12 – irradiated (2 kGy) samples of day 0, 5, 8 and 12).

Analysis of GC spectral data of free aroma by Principal Component Analysis (PCA) revealed changes in the volatile composition as a result of radiation processing (2 kGy) and storage. The control samples at day zero were located in upper left quadrant (negative side of F1 and positive side of F2), while all other samples got segregated from 0 d control samples, and were grouped together. Careful interpretation of GC/MS data revealed quantitative changes in the aroma compounds identified which could be responsible for the segregation in the PC plots.

In case of alcohols, an increased content of 3-methyl-butanol, 2-methyl butanol, hexanol and 2-ethyl hexanol was observed in radiation processed samples on day zero. An increase of 81, 400, 298 and 66 % was observed in the contents of these alcohols respectively as a result of radiation treatment (2 kGy). The glycosidic precursors of these alcohols were also identified in the vegetable. A corresponding decrease in the respective glyco-conjugates was observed. Thus, the increased content of free alcohols in radiation processed samples could be the result of radiolytic breakdown of their glycosidic precursors present in the vegetable. Further, the contents of these alcohols increased during storage in control as well as radiation processed ash gourd. The increased content of various alcohols during storage has also been reported earlier in cantaloupe and watermelon [178]. They correlated increased content of alcohols with senescence of the product. Aroma notes of 3-methyl-butanol, 2-methyl butanol, hexanol and 2-ethyl hexanol are reported to be whisky, wine, sweet-green and citrus/ fresh floral respectively.

Aldehydes, known to impart "green" and "grassy" notes decreased as a result of radiation processing as well as during storage. They are reported to play a relatively important role in vegetable flavors [145]. A decrease of 45 % in the content of 3-methyl butanal, 66 % in 4-methyl-4-penten-2-one, 57 % in 2-pentanone, 35% in hexanal, 41 % in octanal and 62 % in decanal was observed. The C6-C8 aldehydes are known to be formed via the lipoxygense pathway from unsaturated fatty acid precursors namely linoleic and linolenic acids liberated mainly from galactolipids. Decreased content of aldehydes during storage has also been reported earlier in fresh-cut cantaloupe [178]. Reduction of aldehydes to

branched-chain alcohols, and their subsequent conversion to branched-chain acetates was cited as the probable reason by these workers.

Among esters, no significant effect of radiation processing was observed except for ethyl acetate. Ethyl acetate is known to possess fruity odor note, and is widely reported in various fruits and vegetables [191]. Radiation treatment (1 kGy) resulted in 73 % decline in the content of ethyl acetate on day zero as compared to the non-irradiated fresh control samples. Decreased production of esters with increasing radiation dose (0.44 – 1.32 kGy) was also reported earlier in apples stored at 20 °C [211].

A significant decrease in the contents of bound aroma precursors of terpenes was also observed in irradiated samples during storage, indicating breakdown of their glycosidic precursors due to radiation processing and during storage. A maximum decrease of 84 % was observed for glycosidic precursor of sabinene, followed by β-pinene (83 %), indene (79 %), limonene (41 %), and cymene (49 %) with a minimum decrease of 21 % in the case of myrcene. Radiation induced breakdown of terpene glycosides has been widely reported [133, 207-209]. Thus, the decrease in content could be due to radiation induced hydrolysis of glycosidic precursors of these compounds. However, the degradation or oxidation of free terpenes during extended storage could be the reason for the insignificant change observed in their contents in free aroma. Generally terpenes influence the aroma perception of a plant produce significantly. No major changes in the terpene compounds during storage and radiation processing indicates that aroma quality of the vegetable was not altered during the intended storage period (12 d).

A decrease in content of aroma glycosides due to radiation processing has been reported earlier in several food products such as coffee, nutmeg, fenugreek, and papaya [133, 207-209]. In case of nutmeg, glycosidic precursors of p-cymene-7-ol, eugenol, methoxyeugenol and α-terpineol were reported [133]. A radiation dose dependent decrease in content of all glycosides was reported by these authors. In case of fenugreek, radiation dose dependent decrease in content of phenol glycoside was reported [208]. In coffee beans a decreased content of glycosidic precursors of isoeugenol and 4-vinylguaiacol as a result of radiation processing was reported [212]. Thus, our results are in accordance with already published literature data.

Among the key odorants, the content of acetoin increased in radiation treated ash gourd on day zero (C- 12.16 µg/kg; I- 20.39 µg/kg). A decrease by 41 % in the content of octanal was observed, while no significant effect of irradiation was noted in the nonanal content. Although changes were produced by radiation treatment, none were sufficient to be observed in a sensory test. Thus the overall sensory acceptability of the developed product was unaltered with reference to aroma quality.

3.2.5.2. Drumstick

The PC plots for free and bound aroma of drumstick are presented in Figures 42 and 43, respectively. The first two principal components (F1 and F2) explained 30.99 and 22.59 % variation in the data for free aroma and 55.32 and 15.10 % variation in the data for bound aroma.

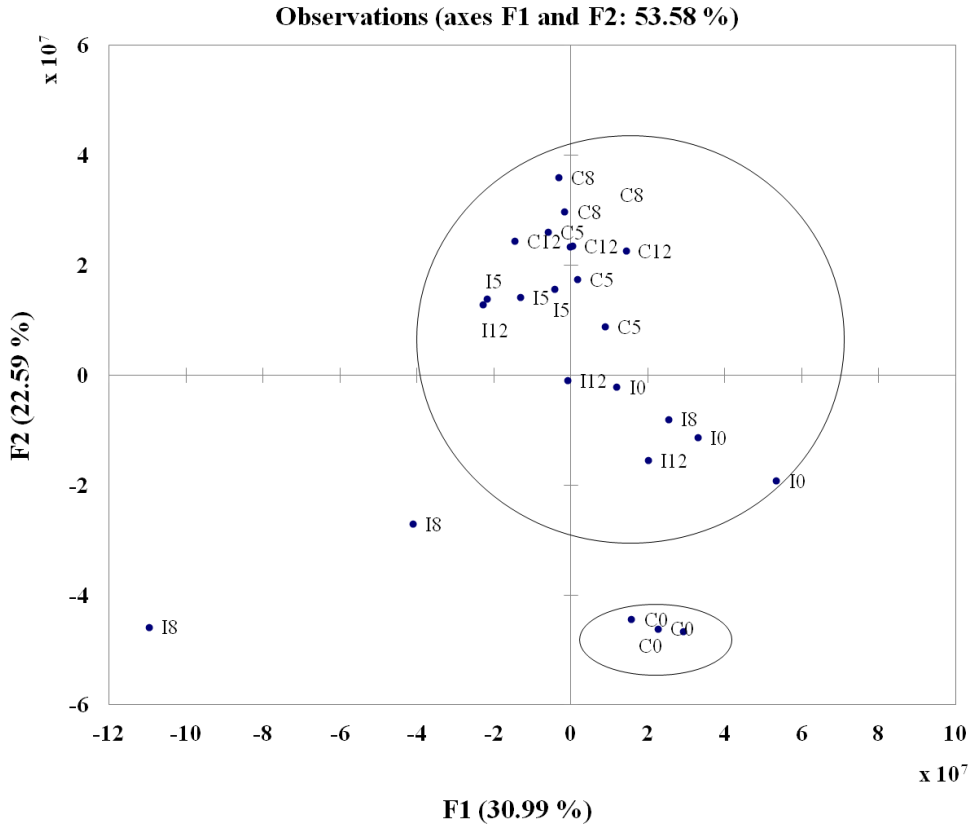


Figure 42. PCA of free aroma of drumstick (C0, C5, C8, C12 – control samples of day 0, 5, 8 and 12; I0, I5, I8, I12 – irradiated (1 kGy) samples of day 0, 5, 8 and 12).

PC plot for free aroma of drumstick shows that fresh control samples (C0) were segregated from all the other samples (Figure 42). However, no clear segregation was observed between the control and radiation processed samples at different storage days, indicating no major changes in the free aroma composition of drumstick. In case of bound aroma, only irradiated samples on day zero were grouped separately, while no variation was observed among other stored samples (Figure 43).

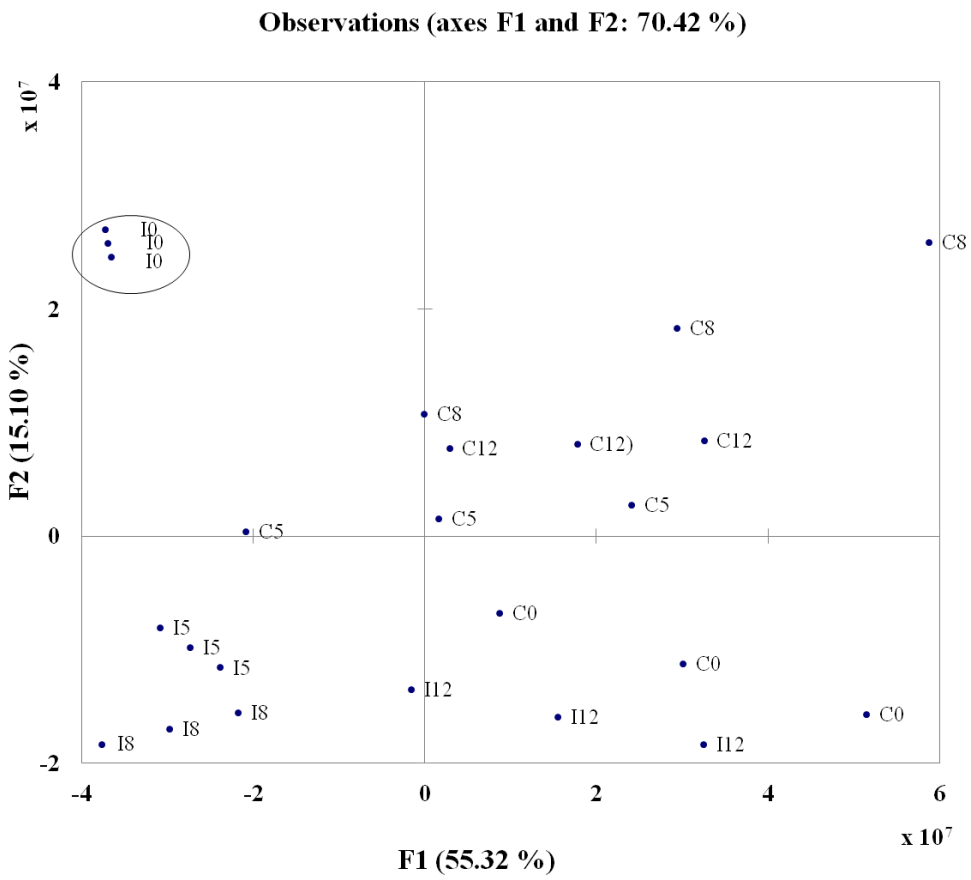


Figure 43. PCA of bound aroma of drumstick (C0, C5, C8, C12 – control samples of day 0, 5, 8 and 12; I0, I5, I8, I12 – irradiated (1 kGy) samples of day 0, 5, 8 and 12)

Careful interpretation of GC/MS data for free aroma compounds revealed some quantitative differences among the compounds identified. Among the aldehydes, a two fold increase in the content of hexanal and 58% increase in the content of trans-2-hexenal were observed as a result of radiation processing. Hexanal is characterized by green, grassy odor note, while trans-hex-2-enal possesses fresh green and leafy aroma. They are reported to play a relatively important role in vegetable flavors [145]. Increased content of these aldehydes due to radiation processing was previously reported in various

vegetables. UV irradiation of tomato fruits and leaves was shown to increase the production of n-hexanal as a result of enhanced lipooxygenase and hydroxyperoxidase lyase activity [213, 214] also reported an increased trans-hex-2-enal content in soybeans due to gamma irradiation at a dose above 10 kGy with as high as 5 times increase at 100 kGy. Fan and Sokorai, on the other hand observed an increase in *trans*-hex-2-enal content of cilantro during post-harvest storage with no significant effect on the content of this compound on irradiation [215]. An increase in the content of trans-2-hexenal was also observed in irradiated cabbage [145]. Thus, increase in trans-2-hexenal might be attributed to radiation induced lipid radiolysis resulting in release of linolenic acid and the subsequent conversion of this fatty acid to this compound via lipooxygenase (LOX) pathway to the aroma compound.

In case of terpenes, α-terpineol exhibited a two fold increase in response to radiation treatment (1 kGy). α-Terpineol is a degradation product of limonene and linalool and rate of formation from linalool is faster than from limonene [216]. A corresponding decrease in content of linalool was also noted after radiation treatment on day zero (control: 2.02 µg/kg; irradiated: 1.13 µg/kg). Although the contents of linalool, limonene and α-terpineol decreased during storage, the content of α-terpineol was higher in irradiated samples than the corresponding controls at the end of intended storage period for 12 d (control: 0.19 µg/kg; irradiated: 0.36 µg/kg).

Various nitrogen and sulphur containing compounds were also detected in the aroma profile of drumstick. The major compounds were 2-methyl propane nitrile, 2-methyl butane nitrile, isopropyl isothiocyanate, 2- butyl isothiocyanate, isobutyl isothiocyanate

and 2-methyl benzonitrile. Many sulphur and nitrogen containing compounds such as glucosinolates have been reported earlier in drumstick [113]. These glucosinolates undergo hydrolysis in the presence of inherently present myrosinase enzyme in the vegetable during storage or processing, giving rise to nitriles, isonitriles, thiocyanates and isothiocyanates. An increase of 16 %, 79 % and 78 % in the content of isopropyl isothiocyanate, 2-butyl isothiocyanate and isobutyl isothiocyanate was observed as a result of radiation processing on day zero. Processing is often associated with increased enzymatic activities, which could have resulted in the increased contents of these components. However, no significant variation in the contents of other compounds was noted in response to irradiation and storage. Although storage caused a decrease in the contents of most of the compounds identified, the contents were significantly higher in radiation processed samples as compared to control samples until the intended storage period of 12 d.

Despite the changes in the contents of various aroma compounds as a result of radiation processing and storage, no significant variation was observed in the contents of key odorants of drumstick. Also, no change in aroma quality was perceived by the sensory panel in irradiated drumstick samples.

Among bound aroma, appreciably more number of alcohols were identified which were, however, not detected in the free aroma. It indicates that most of the alcohols are present in the glycosidic form in drumstick. The major alcohols responsible for the segregation of irradiated samples of day zero (I0) from others in bound aroma were 3-methyl butanol, 2-penten-1-ol, 2-hexanol, 3-hexen-1-ol, hexanol, benzyl alcohol, and α-terpineol. The

content of these compounds in fresh control samples were 12.11 µg/kg, 1.16 µg/kg, 10.77 µg/kg, 24.35 µg/kg, 14.58 µg/kg, 7.34 µg/kg, and 2.44 µg/kg which increased to 38.86 µg/kg, 2.48 µg/kg, 18.92 µg/kg, 35.09 µg/kg, 94.18 µg/kg, 53.94 µg/kg, and 6.24 µg/kg respectively in radiation processed samples. This significant increase could be due to higher extractability of the aroma glycosides in radiation processed drumstick. Such observations have been reported earlier in various food products [217]. Thus the results obtained in present study are in agreement with the already published literature.

The contents of free terpenes released from their glycosidic precursors also increased significantly in response to irradiation. An increase of 82, 300, 90, 82, 33, 131, 68 and 45 % was observed in the contents of citronellol (citrus with green fatty terpene nuances), limonene (citrus), indene (fresh citrus grapefruit), linalool (sweet floral), α-terpineol (pine floral), trans geraniol (floral), myrcene (spicy), and limonene dioxide (citrus) respectively. Greater extractability of the aroma precursors in radiation treated samples might have resulted in the higher amounts obtained. Terpenes generally have substantial influence on the aroma of a product. The increased contents of these aglycones in response to radiation treatment thus suggest improved aroma quality of the product.

3.2.5.3. Pumpkin

Score plots of both the aroma profiles are depicted in Figures 44 and 45, respectively. The first two principal components (F1 and F2) explained 65.4 and 13.9 % variation in the data of free aroma, while 96.4 and 3.1 % variation in the bound aroma was explained by F1 and F2, respectively.

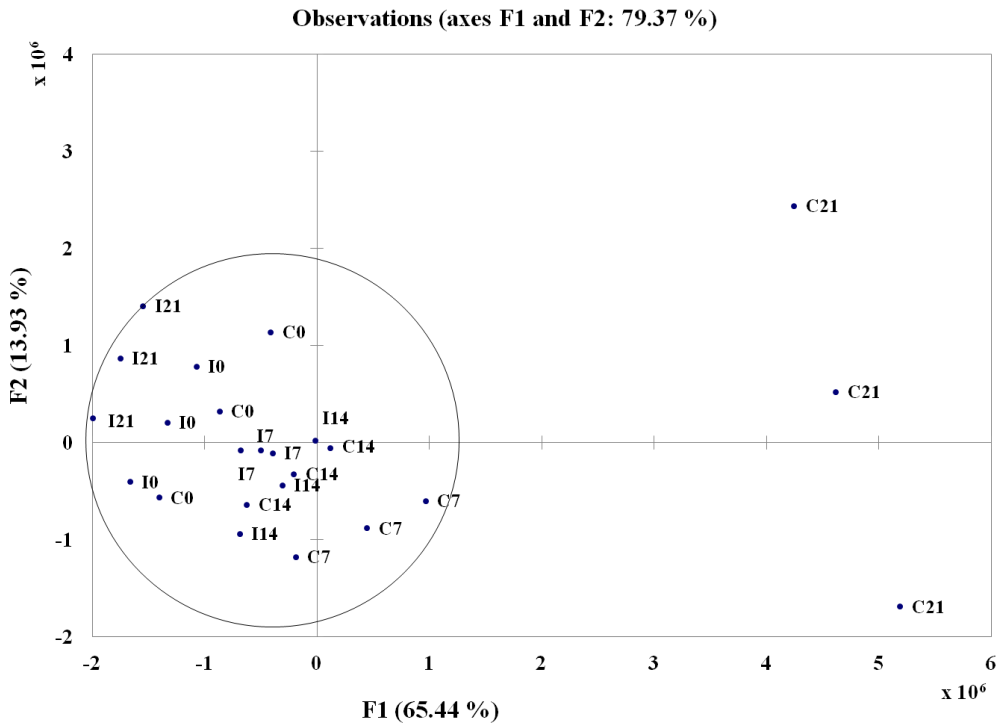


Figure 44. PCA of free aroma of pumpkin (C0, C7, C14, C21 – control samples of day 0, 7, 14 and 21; I0, I7, I14, I21 – irradiated (1 kGy) samples of day 0, 7, 14 and 21).

Score plot of free aroma clearly indicates that there are no major changes in aroma profile due to radiation processing or storage, except for the control samples stored for 21 d, which segregated from the rest of samples. The contents of certain compounds such as acetaldehyde (pungent), 2-methyl-1-propanol (musty), 3-methyl-1-butanol (whisky, malt), 2-methyl butanol (wine, onion), ethanol (sweet) and 3-octanone (musty, mushroom) were found to be higher in control RTC pumpkin after 21 days of storage as compared to other samples. Most of these compounds are reported to be produced by micro-organisms during extended storage due to spoilage [15]. In a study conducted by [17], by inoculating mixed-lettuce agar with spoilage bacteria and yeast, various volatile organic compounds such as ethanol, ethyl acetate, 2-methyl-1-propanol, 2-methyl-1-

butanol, 3-methyl-1-butanol, 2,3-butanedione, 3-methyl-1-pentanol, 1-butanol and 1-hexanol were identified in the head space as spoilage markers. Thus the role of these compounds in contributing to the observed variation in volatile profile of pumpkin samples could be explained.

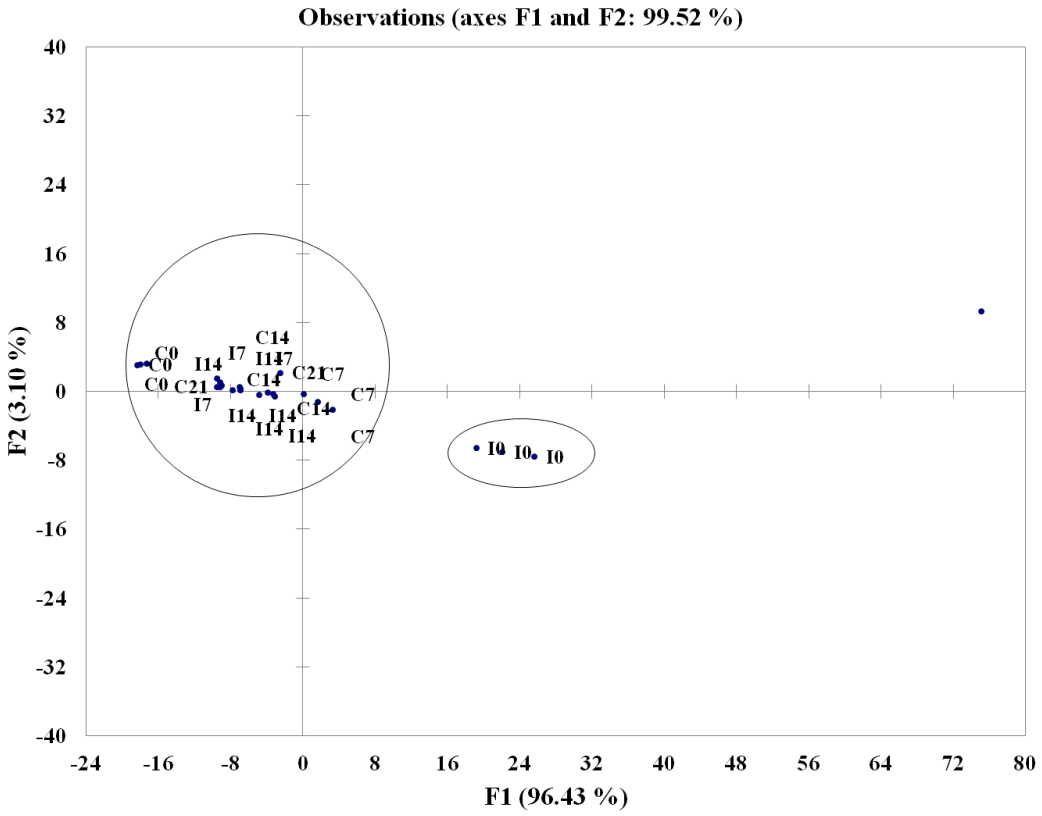


Figure 45. PCA of bound aroma of pumpkin (C0, C7, C14, C21 – control samples of day 0, 7, 14 and 21; I0, I7, I14, I21 – irradiated (1 kGy) samples of day 0, 7, 14 and 21)

Among key odorants, the contents of 6Z-nonenal (C- 2.48 µg/kg; I-4.39 µg/kg) and 2E, 6Z-nonadienol (C-0.27 µg/kg; I-0.52 µg/kg) increased in radiation treated samples. However, their contents were comparable to that of fresh control samples at the end of

storage period. Thus sensory panel could not distinguish between the aroma quality of fresh control and irradiated samples stored for a period of 21 d.

Score plots (Figure 45) for bound aroma indicated that the radiation processed (1 kGy) samples at zero day were different from rest of the samples. By careful interpretation of GC/MS data, this variation was found to be mainly due to the decreased contents of glycosidic precursors of pentanol (C-18.5 µg/kg; I-10.5 µg/kg), Z-3-hexenol (C-7.08 µg/kg; I- 3.63 µg/kg) and hexanol (C-23.49 µg/kg; I-12.06 µg/kg) on day zero. A corresponding increase in the aglycone content was observed in free aroma profile. This could be attributed to radiation induced hydrolysis of aroma glycosides, resulting in increased aglycone content and decreased content of corresponding glycosides. Radiation induced hydrolysis of glycosides has been widely reported in food products resulting in enhanced aroma quality [218]. Thus an overall enhanced aroma quality was observed in radiation processed RTC pumpkin.

3.2.6. Gamma irradiation induced browning inhibition

Studies so far have demonstrated the feasibility of using gamma irradiation for extending shelf life of RTC ash gourd. Irradiated RTC ash gourd had a superior visual appeal compared to the control samples as a consequence of surface browning inhibition (Figure 18). Development of browning in the control samples was also evident with a significantly ($p < 0.05$) lower 'L' value (Figure 15 (a)). Among the physiological factors limiting post harvest storage of fresh plant produce, enzymatic browning plays a major role in reducing sensory quality and nutritional value of these products. Gamma

irradiation induced browning inhibition in cut vegetables has been previously reported by some workers but reports on the mechanism of its inhibition in cut vegetables are scanty.

Browning in cut fruits and vegetables mainly involves metabolism of phenolic compounds into their oxidized products [219]. In intact plants, phenolic compounds in cell vacuoles are spatially apart from the oxidizing enzymes present in the cytoplasm. Once tissues are damaged by cutting, grinding or pulping, the rapid mixing of the enzymes and phenolic compounds as well as the easy oxygen diffusion to the inner tissues results in a browning reaction. In response to tissue injury, phenylalanine ammonia lyase (PAL) produces phenols which are then oxidized by polyphenol oxidase (PPO) and peroxidase (POD) to *o*-quinones that further polymerize to brown pigments.

3.2.6.1. Evaluation of parameters responsible for browning

Control and radiation treated ash gourd samples were processed as detailed in section 2.2.1. Various parameters responsible for enzymatic browning such as electrolytic leaching, changes in micro-structure, enzyme activities (PPO, PAL and POD), phenolic composition and o-quinones were monitored at different storage intervals.

3.2.6.1.1. Electrolytic leaching

Electrolytic leaching signifies the electrolytes leaching out on the surface of cut vegetables due to wounding while processing. The tissues also undergo softening due to hydrolysis of cell wall components (pectin, cellulose and hemicelluloses) during stress (including radiation treatment) and extended storage resulting in increased electrolytic

leaching. The extent of leaching was measured in terms of conductivity and spectrophotometric absorbances at various wavelengths.

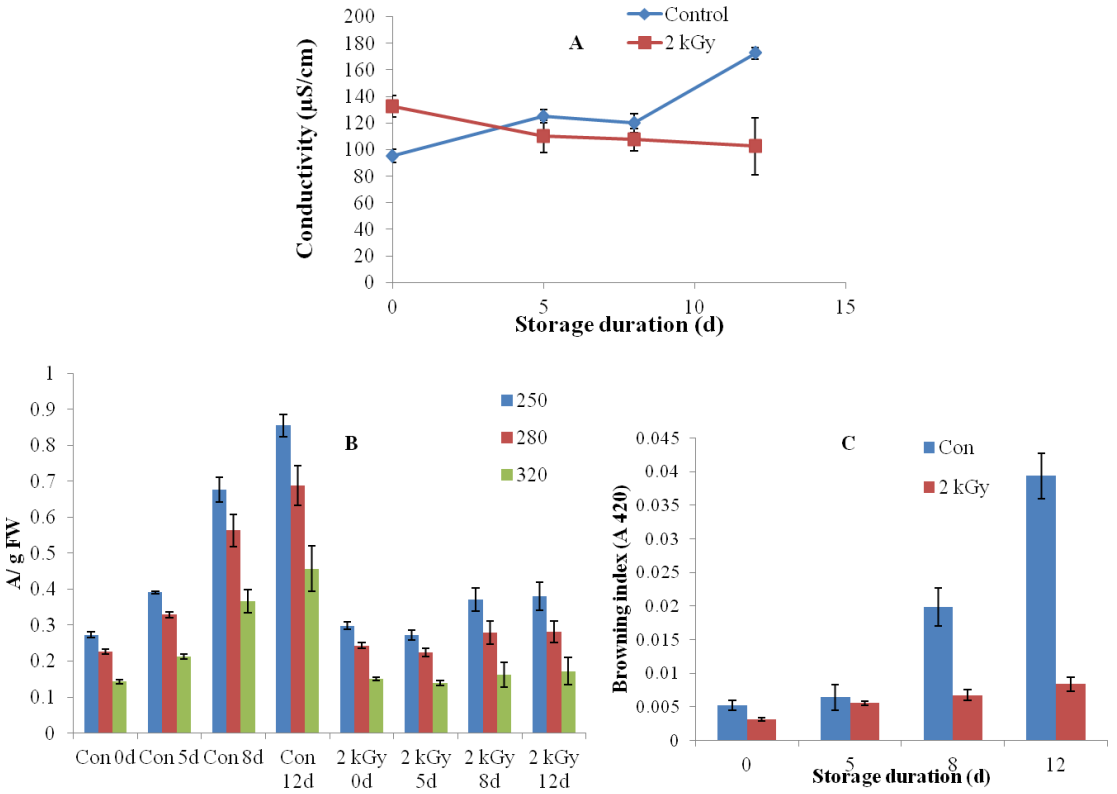


Figure 46. Effect of radiation treatment (2 kGy) and storage on (A) Conductivity (B) Absrbance at 250, 280 and 320 nm (C) Browning index (absorbance at 420 nm) of RTC ash gourd

a) Conductivity: The variation in conductivity of the vegetable extracts with radiation processing during storage is shown in Figure 46 A. A significant increase in values of conductivity was observed in radiation-treated samples on day zero. During storage, the conductivity of control samples increased substantially, while it was unchanged in

irradiated samples. This infers increased content of electrolytes leaching out on the surface of control RTC ash gourd.

b) Absorbance: The absorbance measured at different wavelengths (250, 280 and 320 nm) in control and irradiated samples during storage are shown in Figure 46 B. A significantly higher values of absorbance were obtained in control samples as compared to irradiated during storage. It indicates greater extent of compounds leaching out of the matrix. On the other hand, no change in the absorbance of irradiated samples during storage indicates better integrity of the tissue structure during extended storage. The browning index (absorbance at 420 nm) also increased significantly in control samples on day 8 and beyond (Figure 46 C). The aborbance at 280 nm can be taken as a measure of the phenolic constituents leaching out on the surface. Thus, increased absorbances at 280 and 420 nm indicate more leaching out of the phenolics on the surface of the vegetable, which undergo aerial oxidation in the presence of PPO or POD enzymes, and subsequently form brown pigments at the surface of the control samples. Increase in browning intensity has also been reported earlier in minimally processed lettuce during storage of 5 d [220]. Significantly lower leaching of electrolytes in case of irradiated ash gourd could have resulted in better retention of the visual quality during the intended storage period (12 d).

3.6.1.2. Changes in microstructure

Scanning electron microscopic (SEM) studies demonstrate significant variation in the microstructure of control and radiation treated (2 kGy) samples at the end of storage period (12 d) (Figure 47 A-C). In control samples, after a storage period of 12 d, the

surface microstructure was clearly changed, as indicated by collapsed and damaged tissues ((Figure 47 C). On the other hand, the tissues of radiation processed samples were observed to be undamaged (Figure 47 B). Thus SEM studies further support the findings as observed by electrolytic leaching experiments.

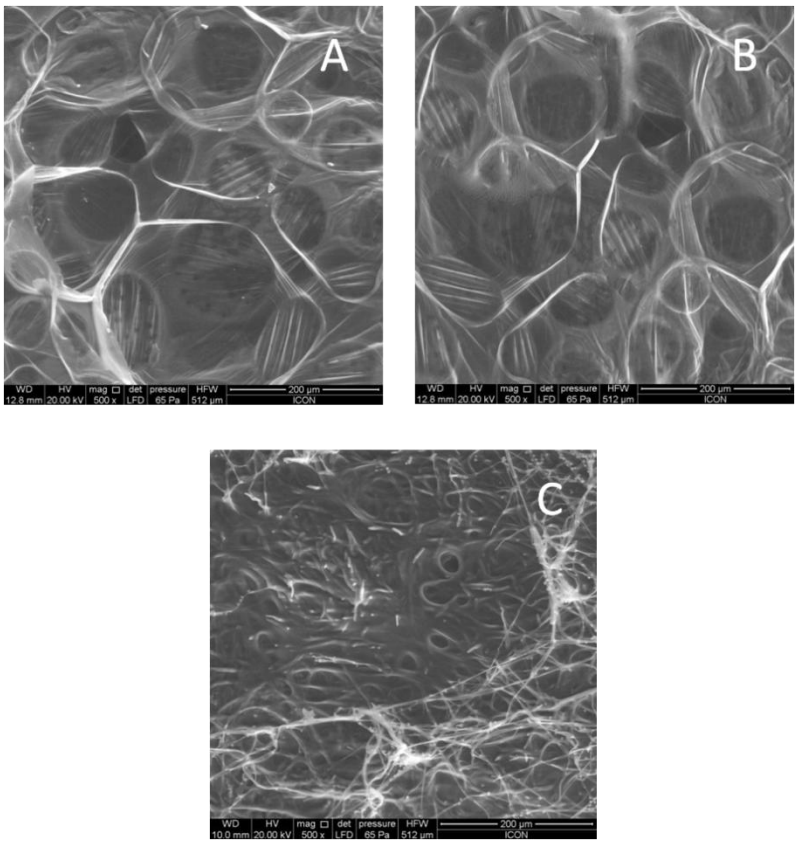


Figure 47. Scanning electron micrographs of RTC ash gourd (A) Non-irradiated control 0 d, (B) Radiation treated (2 kGy, 12 d) and c) Non-irradiated control 12 d. Figures are with 500 x magnification.

3.2.6.1.3. Evaluation of enzyme activities

Alteration in phenolic metabolism is generally known to affect browning in cut vegetables. PAL is the first enzyme in the phenylpropanoid pathway involved in synthesis

of phenolic compounds. Apart from PAL, PPO & POD are the other key enzymes involved in browning development of fresh-cut produce. Figure 48 represents the variation in activities of PAL and PPO with radiation processing and storage. No significant change in the PAL activity was observed as a result of radiation processing on day zero (Figure 48 A). During storage, the PAL activity increased continuously in control samples. Several studies on cut lettuce have shown a wound induced enhancement in PAL activity. Degl Innoceti [138], for instance, noted a significant increase in PAL activity within 5 h, whereas Hisaminato et al [221] found maximum increase after 3 days of storage. Murata et al [27] also found a significant increase in the activity of this enzyme in lettuce after 3 days of storage that further increased on storage up to day 6. Thus, the effect of wounding on PAL activity was found to vary with the variety of lettuce. Stress induced enhancement in PAL activity has been extensively reported in different plant tissues. Various stresses such as nutrient deficiencies, viral, fungi, and insect attack are known to increase either PAL synthesis or activity in different plants [219]. Wound induced enhancement in PAL activity has also been previously reported in minimally processed potatoes [222] and cabbage [145]. In the present case, microbial and physiological spoilage of ash gourd during storage might have induced a stress which resulted in an increase in PAL activity. On the other hand, in radiation processed samples, PAL activity remained unchanged during storage. Induction of PAL is a defence mechanism of the living plant tissues from external stress. Therefore during storage, as the radiation processed samples were protected from spoilage, the metabolic activity of PAL enzyme remained subsided. Higher phenolic biosynthesis as reflected by

increased absorbance at 280 and 320 nm in the control samples also support the pattern of PAL activity observed.

PPO: PPO is a downstream enzyme in the phenylpropanoid pathway acting on phenols to form o-quinone. Radiation induced decrease in PPO activity was observed immediately after treatment (Figure 48 B). Although a gradual increase in the activity was observed during storage in both the samples, the activity of the radiation treated samples was significantly lower ($p < 0.05$) than corresponding control samples throughout the intended storage period (12 d). Similar observations have been reported earlier in edible mushroom (*Pleurotus nebrodensis*), where a significant ($p < 0.05$) radiation induced decrease in PPO activity was observed at a dose of 1.2 kGy [223]. They also observed an increase in the PPO activity in control as well as radiation processed samples during storage, although the activity in controls was higher than the irradiated samples. Duan et al. also reported lowering in PPO activity due to a probable change in the conformation of active sites on exposure to radiation and a consequent inhibition of browning in *Agaricus bisporus* [224]. The results obtained in the present study are thus in accordance with the existing reports.

POD: POD is another enzyme ubiquitously present in plants, that in the presence of hydrogen peroxide converts a number of phenolics to form *o*-quinone. However, its role in enzymatic browning remains questionable mainly because of the low H_2O_2 content in vegetable tissues [219]. Free radicals including H_2O_2 are generated due to water radiolysis on irradiation. Thus analysis of POD activity is of significance in the present study. POD activity was assayed in the presence of natural hydrogen donors (chlorogenic

and caffeic acid). POD activities did not vary substantially during storage for both the substrates (Table 37), thus ruling out its role in browning in RTC ash gourd.

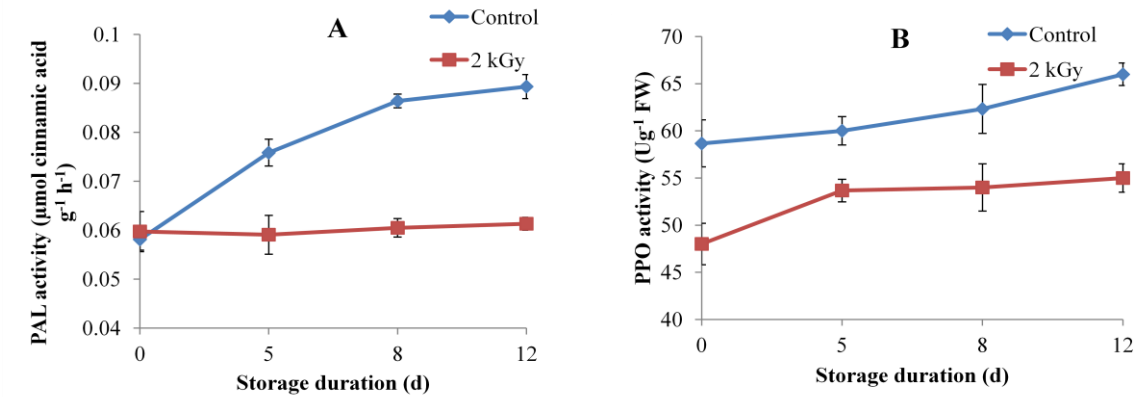


Figure 48. Effect of radiation processing (2 kGy) and storage on (A) PAL activity and (B) PPO activity

Table 37. Effect of radiation processing (2 kGy) and storage on POD activity of ash gourd

Day	Control		Irradiated (2 kGy)	
	POD1	POD2	POD1	POD2
0	7.25 ± 1.25^{a}	5.65 ± 1.05^{b}	5.55 ± 0.55^{a}	4.85 ± 1.05^{b}
5	7.00 ± 0.95^{a}	5.3 ± 1.25^{b}	6.25 ± 0.90^{a}	4.9 ± 0.99^{b}
8	6.05 ± 0.80^{a}	4.95 ± 0.95^{b}	5.75 ± 1.10^{a}	5.2 ± 0.75^{b}
12	5.75 ± 0.70^{a}	4.35 ± 0.55^{b}	5.1 ± 0.85^{a}	5.05 ± 0.55^{b}

Data are expressed as mean ± standard deviation (n = 9). Mean values in the same column bearing same superscript shows no significant difference ($p \leq 0.05$). POD activity is represented in Δ A min^{-1} g^{-1} FW; POD1 = chlorogenic acid peroxidase activity and POD2 = caffeic acid peroxidase activity.

3.2.6.1.4. Analysis of phenolic constituents

Phenolic compounds are the primary substrate for the enzymes involved in browning, and also their nature and content plays crucial role in browning development of fresh-cut produce. The nature of phenolic constituents and their quantitative distribution during radiation processing and storage was therefore analyzed in RTC ash gourd. The major phenolic constituents identified in ash gourd included phenolic acids (α-resorcylic, p-hydroxy benzoic, chlorogenic, syringic, p-coumaric acid) along with catechin and naringenin (Table 38). As development of browning started on day 8, phenolic contents were compared only from day 8 onwards. The contents of *p*-hydroxy benzoic, chlorogenic, caffeic, and *p*-coumaric acid were significantly higher ($p < 0.05$) in control samples as compared to the corresponding irradiated products on day 8 and 12 (Table 38). The increased content can be attributed to higher PAL activity as observed in control samples during storage (Figure 48 A). These acids are highly prone to oxidation and give rise to brown/black polymers during aerial oxidation in the presence of PPO or POD. Thus, their higher content in the control samples could explain the browning of these samples towards the end of the intended storage period.

No significant effect of radiation processing was observed on the contents of most of the phenolic constituents in ash gourd, except chlorogenic and caffeic acid, which exhibited significant decrease ($p \leq 0.05$) (Table 38). Interestingly, a radiation induced enhancement by 87 % in the content of α-resorcylic acid was observed on day zero. An enhanced synthesis of phenolic acids such as benzoic and cinnamic acids as a result of increased PAL activity under stress in plants has been established earlier [225]. The content of

resorcylic acid was significantly higher in irradiated samples as compared to controls on each day of storage (Table 38). The acid is known to be a potential inhibitor of PPO. Billaud et al. have reported an inhibition in browning of gum Arabic as a result of inhibitory activity of α-resorcylic acid towards PPO isolated from this exudates [226]. The increased resorcylic acid content in irradiated RTC ash gourd could thus possibly explain the prevention of browning in the treated product.

Table 38. Quantitative distribution of different phenolic constituents in ash gourd

	Con 0d	Con 5d	Con 8d	Con 12d	2kGy 0d	2kGy 5d	2kGy 8d	2kGy 12d
α-Resorcylic acid	26.45 ± 2.59^{a}	27.35 ± 2.98^{a}	19.29 ± 2.01^{ab}	7.1 ± 1.07^{c}	49.5 ± 3.97^{d}	46.87 ± 4.22^{d}	43.74 ± 3.65^{d}	18.74 ± 2.12^{a}
Catechin	18.15 ± 1.97^{a}	24.44 ± 2.55^{ab}	24.91 ± 2.98^{ab}	25.59 ± 3.01^{ab}	15.85 ± 1.59^{a}	16.66 ± 1.12^{a}	16.93 ± 1.33^{a}	20.22 ± 1.17^{a}
p-Hydroxy benzoic acid	18.62 ± 1.33^{a}	18.93 ± 1.12^{a}	24.09 ± 1.97^{ab}	24.18 ± 1.87^{ab}	16.08 ± 2.01^{a}	15.79 ± 1.09^{a}	15.68 ± 1.12^{a}	15.95 ± 0.94^{a}
Chlorogenic acid	37.25 ± 3.37^{a}	39.08 ± 3.14^{a}	39.87 ± 3.01^{a}	38.03 ± 2.66^{a}	15.74 ± 1.17^{b}	16.13 ± 1^{b}	15.51 ± 1^{b}	17 ± 1.23^{b}
Caffeic acid	10.03 ± 1.26^{a}	12.64 ± 1.17^{a}	18.58 ± 1.92^{ab}	24.99 ± 2.02^{b}	5.44 ± 0.56^{c}	6.83 ± 0.6^{c}	4.31 ± 0.44^{c}	6.08 ± 0.46^{c}
Syringic acid	79.41 ± 7.53^{a}	73.95 ± 8.52^{a}	70.87 ± 7.8^{a}	58.21 ± 5.23^{ab}	77.35 ± 7.71^{a}	71.03 ± 6.96^{a}	63.59 ± 5.84^{a}	47.75 ± 3.97^{b}
p-Coumaric acid	15.42 ± 1.57^{a}	20.49 ± 2.37^{a}	24.47 ± 2.22^{ab}	26.29 ± 2.33^{ab}	11.79 ± 0.97^{c}	11.79 ± 0.93^{c}	11.72 ± 0.69^{c}	14.71 ± 0.89^{c}
Ellagic acid	1.23 ± 0.22^{a}	1.27 ± 0.2^{a}	1.62 ± 0.33^{a}	1.64 ± 0.33^{a}	1.22 ± 0.22^{a}	1.68 ± 0.22^{a}	1.72 ± 0.45^{a}	1.53 ± 0.37^{a}
Naringenin	2.21 ± 0.31^{a}	1.98 ± 0.21^{a}	1.9 ± 0.33^{a}	1.72 ± 0.16^{a}	3.13 ± 0.34^{a}	3.47 ± 0.36^{a}	3.82 ± 0.3^{ab}	4.04 ± 0.54^{ab}

Data are expressed as mean ± standard deviation (n = 6). Mean values in the same row bearing same superscript shows no significant difference ($p \leq 0.05$).

3.2.6.1.5. Correlations between various factors contributing in browning inhibition

Correlation coefficients were calculated between various attributes involved in browning phenomenon (Table 39). A high correlation between browning index (BI) and enzyme activities (PAL and PPO) was noted in the control samples, indicating their significant roles in browning. A positive correlation between browning and PPO activity has also been reported earlier in peaches [227]. A moderate to high correlations were also obtained between BI and contents of various phenolic acids (caffeic, chlorogenic and p-coumaric acid). Thus an enhanced content of these acids could result in browning during storage. Further, a negative correlation between BI and α-resorcylic acid content and between the PPO activity and α-resorcylic acid provides evidence for the decreased PPO activity in irradiated samples as a consequence of the increased content of this phenolic acid. Therefore, its role in PPO inhibition and subsequently in browning inhibition during storage was further investigated.

Table 39. Correlation coefficients calculated between various factors contributing in browning of RTC ash gourd

S.No.	Parameters	Correlation coefficient (R)
1.	BI and PAL activity	0.93
2.	BI and PPO activity	0.92
3.	BI and Caffeic acid	0.98
4.	BI and Chlorogenic acid	0.96
5.	BI and p-Coumaric acid	0.96
6.	BI and α-resorcylic acid	-0.82
7.	PPO and α-resorcylic acid	-0.97

3.2.6.1.6. Role of α-resorcylic acid in inhibition of ash gourd PPO - Kinetics studies

A resorcylic acid concentration dependent decrease in browning was observed when catechol was used as substrate (Figure 49 A). This observation demonstrated the role of α-resorcylic acid in browning inhibition. The nature of inhibition and kinetic parameters of enzyme inhibition were therefore further investigated.

The type of inhibition was deduced from Lineweaver-Burk (LB) double-reciprocal plot (Figure 49 B). The linear plots obtained between 1/S and 1/V at different concentrations of resorcylic acid were found to intersect on the left of the vertical axis and above the horizontal axis. A decrease in Vm and an increase in Km was thus noted with increasing concentrations of resorcylic acid. Thus inhibitory mode of resorcylic acid was found to be of mixed type as both- slope as well intercept of the LB plot changed with different

concentrations of the inhibitor. This type of inhibition indicates that resorcylic acid affected the affinity of the enzyme for catechol but did not bind at the active site of the enzyme.

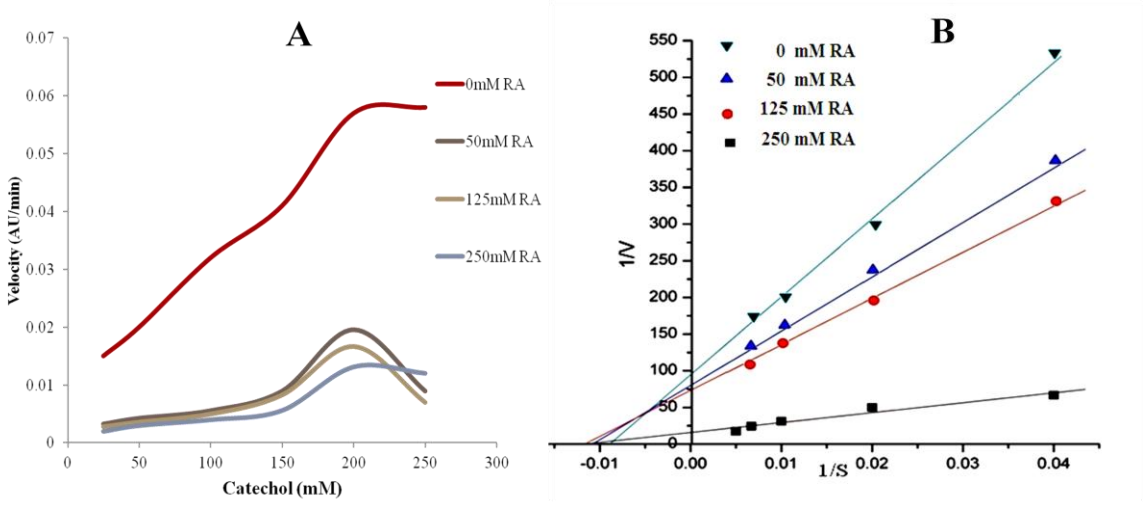


Figure 49. (A) Effect of resorcylic acid (0, 50, 125 and 250 mM) on the browning reaction rate of catechol. (B) Lineweaver-Burk plot of resorcylic acid inhibition on the catechol-ash gourd PPO system

The K_i (rate constant for reaction between enzyme (E) & substrate (S)) and K_{is} (rate constant for reaction between enzyme-sbustrate complex (ES) & inhibitor (I)) values were determined from the graphs between 1/Vmax vs [I] and Km/Vmax vs [I] respectively (Figure 50 A & B). The value of $K_i << K_{is}$, implied a competitive inhibition, with higher affinity of inhibitor for free enzyme rather than ES complex. In inhibition studies of α-resorcylic acid on PPO from gum Arabic, a quasi-noncompetitive inhibition with K_i value around 5 times lower than K_{is} value was noted [228]. Inhibitory effect by various phenolic acids on PPO from various sources have been reported [228, 229]. PPO from different sources has been shown shown to be inhibited by phenolic

compounds via different mechanisms. To the best of our knowledge, this is the first report on inhibition of ash gourd PPO by α-resorcylic acid.

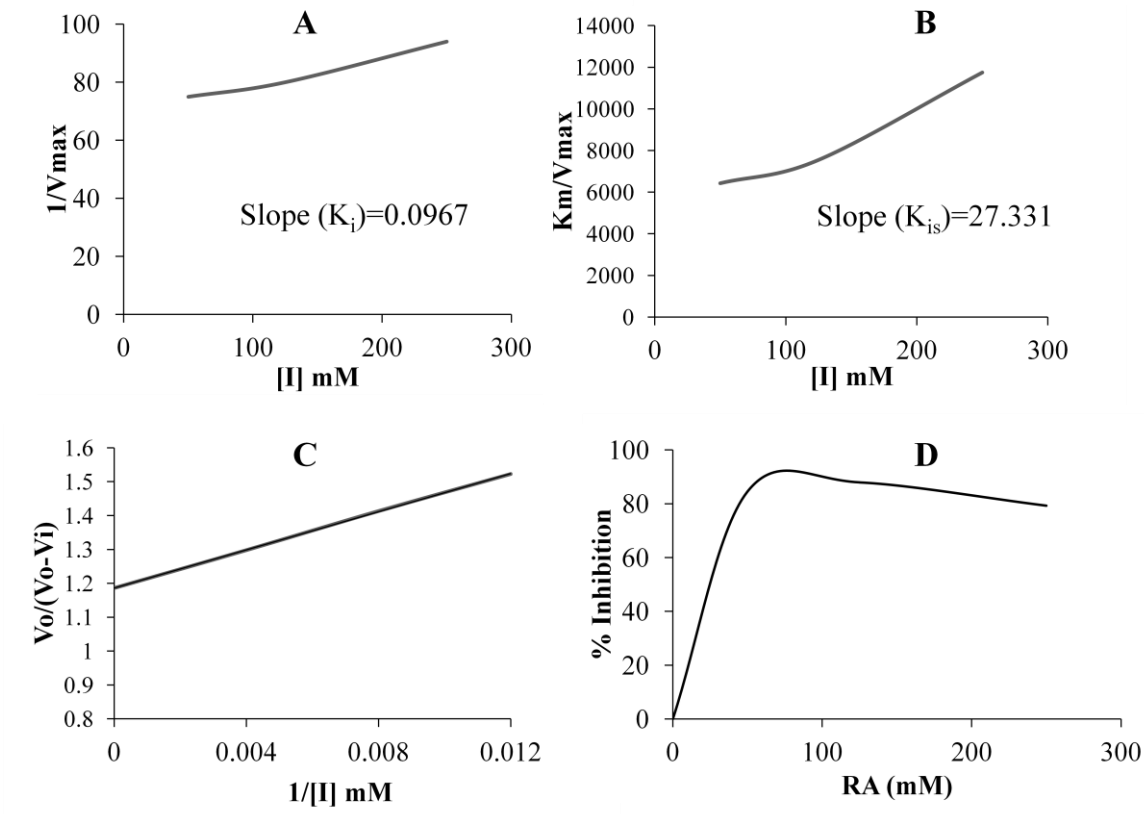


Figure 50. Plots for determining various kinetic parameters. (A) Plot of 1/Vmax vs [I]; (B) Plot of Km/Vmax vs [I]; (C) Plot of $V_0/(V_0-V_i)$ vs 1/[I] (where V_0 is the initial velocity without inhibitor and V_i is the initial velocity in the presence of inhibitor); (D) Inhibitory effect of different concentrations of α-resorcylic acid on the oxidation of catechol by ash gourd PPO.

The reversible nature of inhibition of ash gourd PPO by α-resorcylic acid using catechol as substrate was demonstrated from a plot of $V_0/(V_o-V_i)$ vs 1/[I] (Figure 50 C; V_0 –

velocity of reaction in absence of inhibitor; Vi – velocity of reaction in presence of inhibitor). An intercept value > 1 on Y-axis implied reversible nature of inhibition. The IC_{50} value was determined by a calibration graph between percent inhibition and [I] (Figure 50 D). At a concentration of 25 mM, α-resorcylic acid exhibited 50 % inhibition in the ash gourd PPO activity.

Thus the role of α-resorcylic acid in PPO inhibition was demonstrated. The nature of inhibition was found to be mixed type, competitive and reversible, with IC_{50} value of 25 mM. Hence, radiation induced enhancement in the content of resorcylic acid could contribute towards PPO inhibition, and thus preventing browning in radiation processed RTC ash gourd.

3.3. Isolation, identification of bioactive constituents and determining their changes during radiation treatment

There is an increasing interest in identifying bioactive components in native plant foods and determining their role in contributing to the functional and nutraceutical properties of these foods. This has resulted in concerted efforts towards identifying such foods possessing novel bioactive organic molecules. Indian vegetables have traditionally been used as medicinal ingredients in Ayurveda due to their unique bioactivities. Despite being a treasure house of active constituents, very few studies exist on the nature of the constituents present in majority of the Indian vegetables.

3.3.1. Ash gourd

Ash gourd (*Benincasa hispida*) is known for its medicinal values and healing power and has been widely used as a vegetable as well as a traditional medicine in the orient. As a rich source of functionally important bioactives and therapeutics such as triterpenes, phenolics, sterols and glycosides, the fruit has been widely used for the treatment of epilepsy, ulcer, and other nervous disorders in the traditional medicinal systems of Asia. The juice of the vegetable is recommended to patients suffering from heart ailments and high blood pressure. Ash gourd waste (peel and seeds) has been traditionally used as a green manure for increasing the soil nutrients while shelled seeds are reported to have anabolic properties that promote tissue growth. The vegetable extract was therefore screened for the bioactive principles responsible for some of the above activities.

3.3.1.1. Plant growth promoting activity

Restriction on the application of chemicals to enhance plant growth due to their negative effects has resulted in increased interest in the isolation of novel natural plant growth promoters. This has led to the identification of several newer plant growth regulators such as jasmonates, brassinosteroids, salicylic acid, plant peptide hormones, polyamines, nitric oxide, strigolactones and karrikins that influence various physiological processes such as rooting, flowering, senescence, organogenesis or growth. In recent years, airborne volatiles identified as acetoin and 2,3-butanediol from growth promoting strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens* have also been shown to stimulate growth of *Arabidopsis thaliana* [230-232]. Microbial and plant derived compounds with

bio-regulatory activity thus represent a large pool of chemicals that show promise for development of novel agrochemical compounds. Acetoin was found to be a major aroma compound existing both in the free as well as in the bound form of the vegetable. As this compound has been earlier shown to have growth promoting activity, it was of interest to understand its role in the known growth promoting activity of the vegetable.

3.3.1.1.1. Isolation and identification of plant growth regulating compound

The total aqueous extract of ash gourd was fractionated into non-polar, medium polar and polar fractions by using *n*-hexane, ethyl acetate and *n*-butanol, respectively. Except *n*-butanol extract, all other fractions including the remaining aqueous residue had no growth promoting activity. The butanol fraction was therefore used for subsequent isolation of the active principles. Presence of activity in the n-butanol fraction suggested a polar nature of the active compound(s). When subjected to TLC, the butanol fraction resolved in five distinct bands (Rf values of 0.38, 0.44, 0.52, 0.59 and 0.77), while no clear separation could be observed in the total aqueous extract. The component(s) present in the major band at R_f 0.44 alone exhibited growth promoting activity. Acid hydrolysis of the methanol eluate of this band and subsequent GC/MS analysis of the ether extract of the hydrolyzate resulted in the identification of acetoin as its sole constituent. Analysis of the acetylated derivative of the hydrolyzate remaining after diethyl ether extraction indicated the presence of glucose thus suggesting the major active constituent to be acetoin glucoside. The structure of the compound (TLC band, Rf 0.44) was further confirmed by IR and NMR spectral analysis (Figure 51). The absorption band at 1665 cm^{-1} in IR spectrum indicated the presence of carbonyl group in the molecule. The NMR signals at δ

2.01 and 4.22 confirmed the presence of keto methyl and oxy methine group while a doublet at δ 1.30 was assigned for the terminal methyl group in the acetoin moiety. A broad signal in the range of δ 4.5-5.2 corresponding to sugar protons confirmed the presence of carbohydrate moiety in the molecule. The IR absorption band at 910 cm^{-1} and NMR peak at δ 4.2 (anomeric proton) suggested β- nature of the glycosidic linkage. The β-linkage of the glucosidic bond was further confirmed based on the ability of commercially available β-glucosidase enzyme to hydrolyze purified natural acetoin glucoside. The chemical structure of the acetoin glucoside was further substantiated by its chemical synthesis. Thus the structure of the molecule was identified as acetoin-β-glucopyranoside.

3.3.1.1.2. Growth promotion studies

The effect of *in vitro* treatment of total aqueous extract of ash gourd, acetoin glucoside (TLC isolated band) and standard acetoin at different concentrations (0.178 – 4.4 µg/mL) on the diameter of tobacco leaf discs on day 14 is presented in Figure 52A. A significant increase ($p \leq 0.05$) in the leaf disc diameter was observed in all the treatments compared to the control. Highest growth promotion activity was noted with acetoin glucoside followed by acetoin and aqueous extract. An increase in diameter was observed up to a concentration of 0.44 µg/mL in all the treatments. Beyond this concentration the diameter was higher than the control, but lower than that observed at concentrations of 0.44 µg/mL in all the cases. Thus, an optimum concentration of 0.44 µg/mL was inferred. A toxic effect on the leaves could possibly explain the retardation in growth of the leaf

discs beyond this concentration. At a concentration of 4.4 µg/mL, complete inhibition in growth was observed for all the treatments.

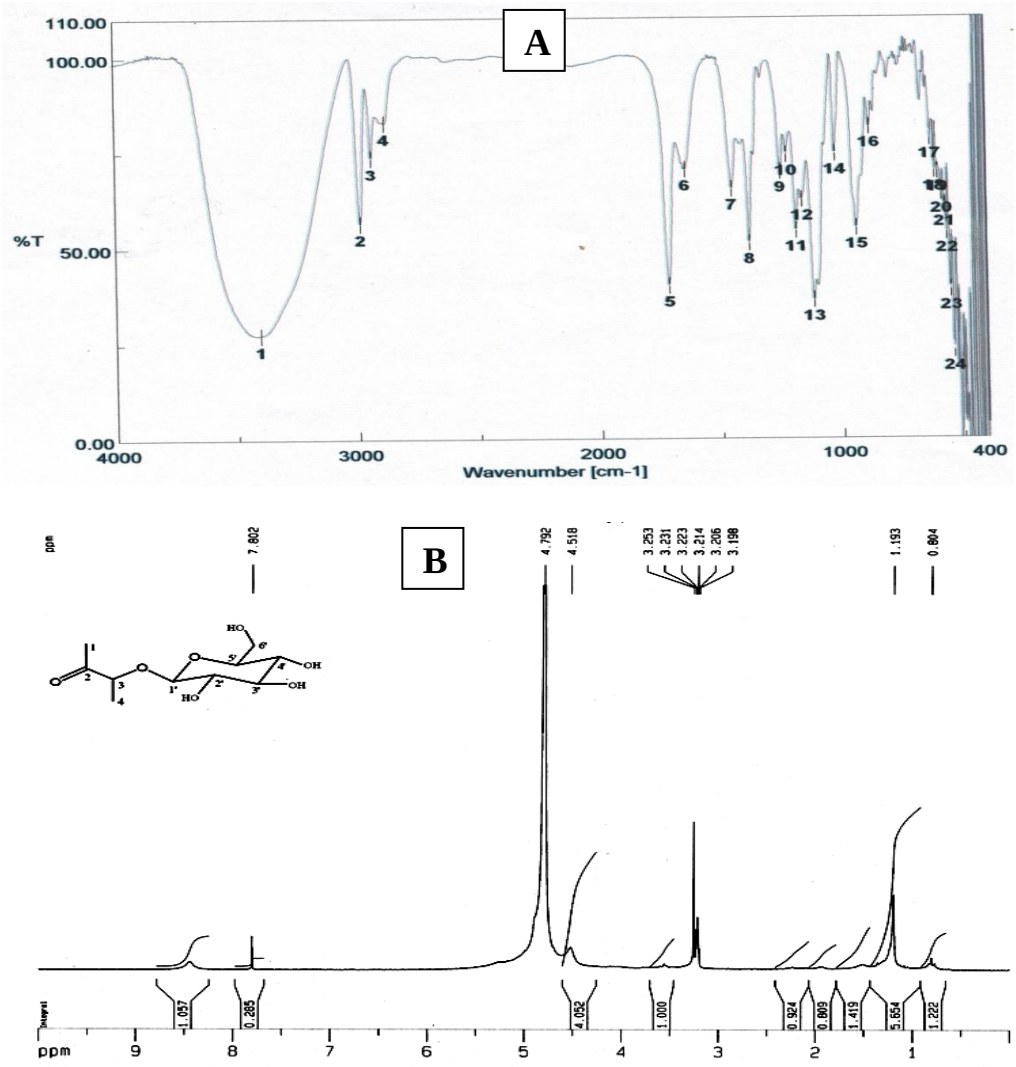


Figure 51. IR and NMR spectra of acetoin-3-O-β-D glucoside

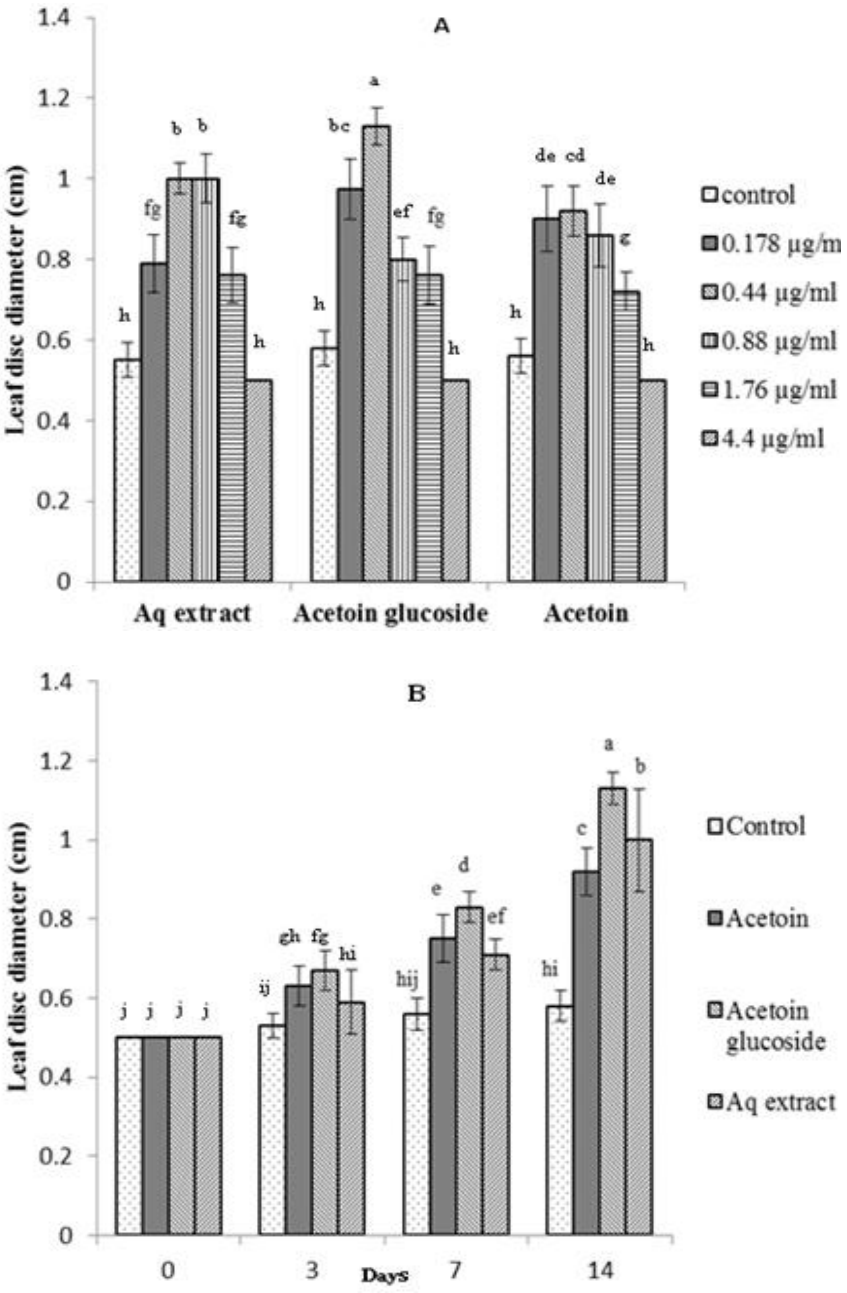


Figure 52. A) Effect of varying concentration (0.178 – 4.4 µg/mL) of total aqueous extract (Aq extract) of ash gourd, acetoin glucoside and acetoin on tobacco leaf disc diameter on day 14 (Different letters indicate significant differences) B) Effect of the various treatments at the optimum concentration (0.44 µg/mL) on the diameter of leaf discs during different time intervals (Different letters indicate significant differences).

Figure 52B shows the effect of various treatments at the optimum concentration (0.44 µg/mL) on the diameter of leaf discs during different time intervals. A significant increase ($p \leq 0.05$) in diameter by 44 % (0.9 cm), 56 % (1.13cm) and 50 % (1.0 cm) was noted in leaf discs treated with acetoin, acetoin glucoside and total aqueous extract respectively on 14 d. In contrast, diameter of control leaf discs remained almost constant during the entire time period studied. Beyond 14^{th} day, the leaf discs underwent decay and hence the experiments were not continued further. Acetoin glucoside was found to induce maximum growth response. This could possibly be due to the ready uptake of the glucoside in comparison to its aglycone by the leaf discs. Figure 53A depicts the enhancement in leaf disc diameter by acetoin glucoside at the optimum concentration of 0.44µg/mL on day 14. In a study on the growth promoting activity of volatile compounds produced by bacterial strains GB03 and IN937a, Ryu et al [231] found that concentration of acetoin released by these bacteria over a period of 24 h were 12 ± 5 µg and 8.8 ± 2.2 µg respectively, while concentration of 2, 3-butanediol was reported to be 3.9 ± 0.7 and 1.9 ± 0.5 µg, respectively. Induction of growth at lower concentrations in the present study could be due to the greater uptake of these compounds by the leaf discs during soaking unlike earlier studies on PGPRs, where restricted diffusion of volatiles into the leaf from the atmosphere reduces this effect. The present study thus demonstrates that acetoin and its glucoside possesses growth promoting activity.

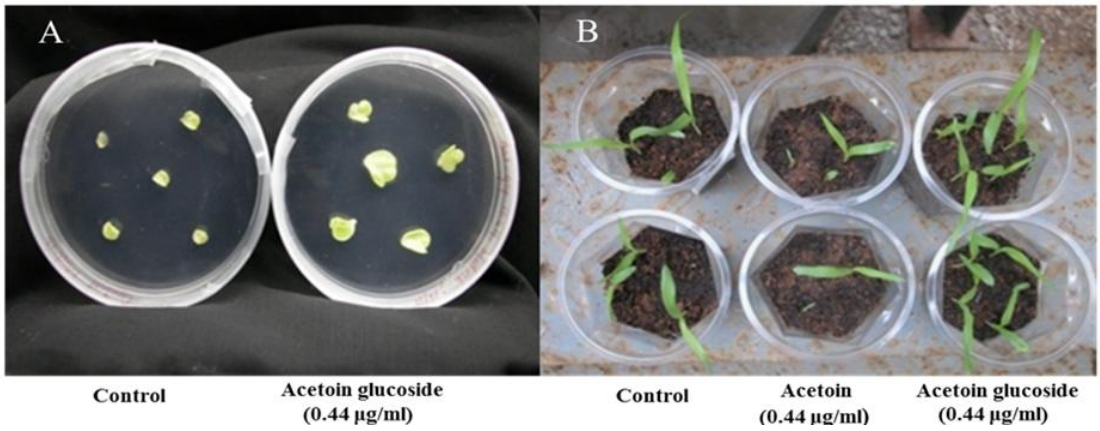


Figure 53. A) Increased leaf disc diameter for acetoin glucoside (0.44 µg/mL) treatment on day 14. B) Germination in pearl millet seeds after treatment with acetoin and its glucoside

In order to further confirm the plant growth promoting activity of acetoin and its glucoside, pearl millet seeds were soaked in acetoin and acetoin glucoside (0.44 µg/mL each) overnight and then sowed in pots (5 seeds per pot) containing autoclaved soil and cocopet (1:1). Seeds soaked overnight in sterile distilled water were used as control. The treatment by acetoin glucoside enhanced leaf length (33.5 ± 3.3 cm) and shoot length (5.5 ±1.5 cm) when the seeds were allowed to germinate and grow for a period of 15 days (Figure 53B). In contrast, the control and acetoin treated samples had similar leaf and shoot length (25.5 ± 2.6 cm and 2.8 ± 0.8 cm respectively). High water solubility of acetoin glucoside unlike acetoin could explain the observed enhancement in plant growth when treated with the former. It is likely that once absorbed into the seed, the glycoside could be hydrolyzed to acetoin that then promotes germination and growth. This however needs further confirmation.

3.3.1.1.3. Identification of differentially expressed protein in acetoin glucoside treated tobacco leaf discs

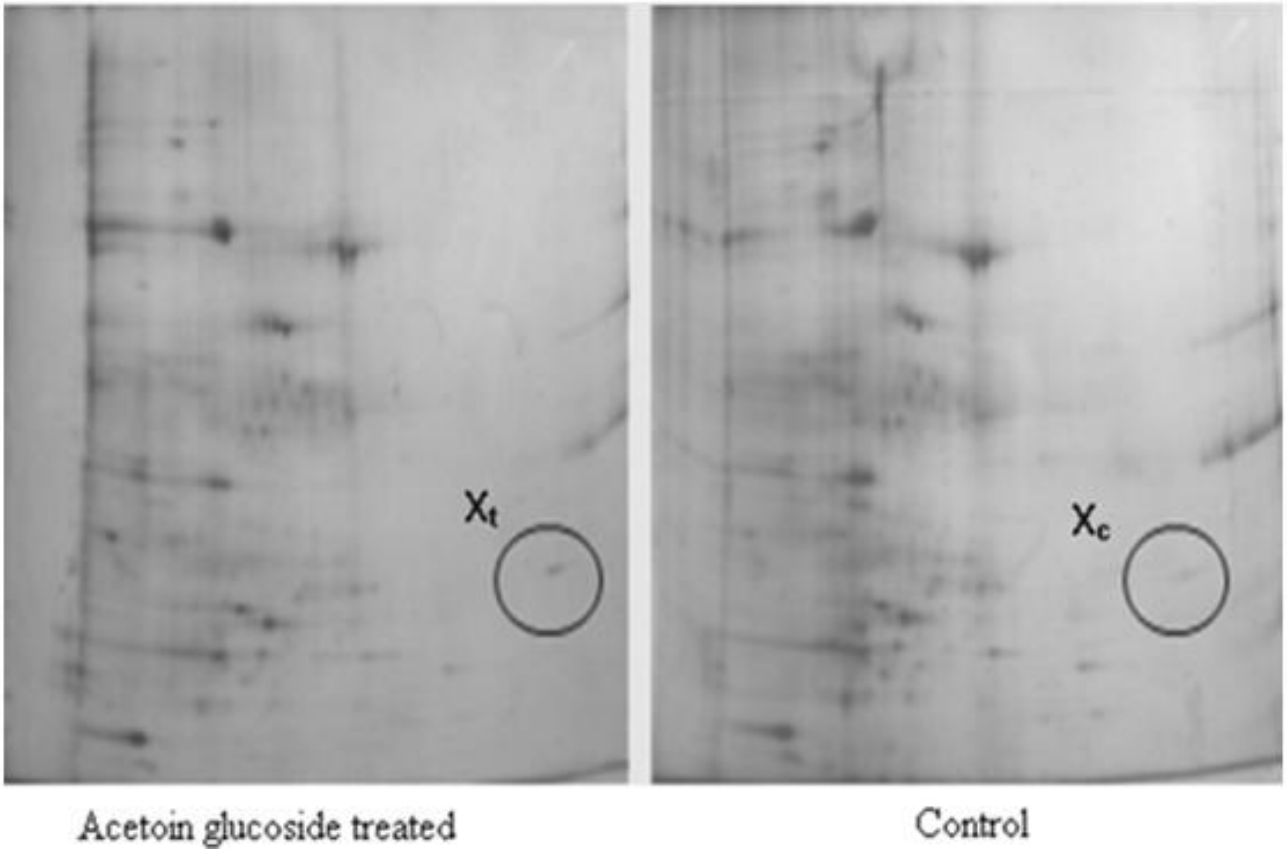


Figure 54. Two dimensional gel from protein preparations of control and acetoin glucoside treated tobacco leaves; X_t- protein over expressed in treated sample, X_c – corresponding protein in control sample.

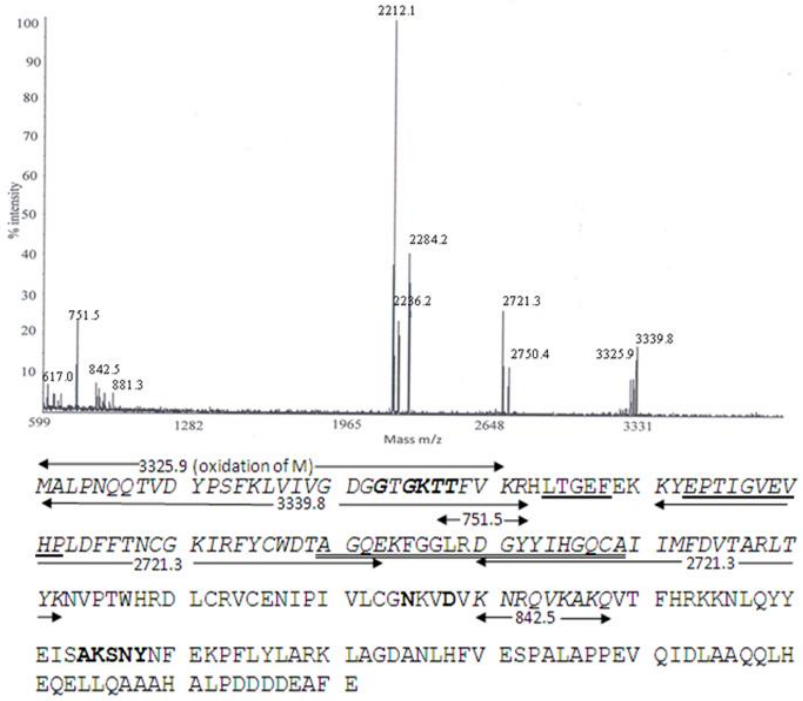


Figure 55. Mass spectrum obtained by MALDI-ToF analysis of the protein (X_t) over-expressed in response to acetoin glucoside treatment.

Figure 54 depicts the 2-D gel electrophoresis images of control and acetoin glucoside treated tobacco leaves. In the present study we report the identification and bioinformatic characterization of a protein (X_t) expressed in response to acetoin glucoside treatment. Analysis of this protein by MALDI-TOF provided information on the peptide molecular mass. The mass spectrum of this protein is shown in Figure 55. Searching the MASCOT database yielded the best hit as nuclear GTP binding protein RanB1 in tobacco (*Nicotiana tabacum*; Swiss Prot accession No. P41919). The best fit peptide masses in tobacco Ran B1 are shown in Figure 55. Ran is a small nuclear protein (25 kD) with multiple

regulatory roles [233]. Evolutionarily conserved Ran belongs to Ras superfamily and has been demonstrated to be a very important nuclear transport regulator [234]. Ran toggles between GTP and GDP bound forms. A GTP bound form is activated for its GTPase function by RanGAP protein while a new GTP molecule replaces the bound GDP by the action of RanGEF [235]. There are local concentration variation of these two Ran modifying proteins within the cell causing concentration gradient of RanGTP and RanGDP [236]. This concentration gradient is believed to be important for the regulatory functions of Ran in nuclear transport. It also plays a very important role in DNA replication and cell cycle progression [237]. It regulates the spindle assembly before chromosome separation, and also the nuclear envelop formation after mitotic chromosome separation is completed. Overexpressed Ran1 in transgenic Arabidopsis and wheat showed increase in cell number [238]. Transgenic arabidopsis showed distinct phenotype such as increased tiller number, weak apical dominance, excess rosette leaves, and wider siliques. Therefore, from studies carried out by these researchers, it is evident that Ran is an important protein for cell division and tissue growth. As observed in our experiments, acetoin and its glucoside from ashgourd increased the biomass production. We find the presence of RanB1 among the upregulated proteins quite significant. However it is unclear whether acetoin and/or its metabolic derivative(s) directly work on Ran up-regulation or other regulatory proteins are also involved. Ran and its regulatory functions are at the cross roads of many biochemical pathways. Observation of Ran up-regulation in response to ashgourd derived acetoin glucoside treatment sheds a new light on the mechanism of acetoin mediated growth enhancement. Currently our efforts are

concentrated on identifying the other overexpressed proteins in response to treatment of plant tissue with acetoin glucoside. This will provide a greater insight into the mechanism of acetoin action. The treatment of plants with highly active but cheap chemicals such as acetoin and acetoin glucoside could have potential use in increasing crop yield.

3.3.1.2. Angiotensin converting enzyme (ACE) inhibition activity

Ash gourd has been used in traditional Indian and Chinese medicine to treat inflammation and high blood pressure. Its juice is recommended to patients suffering from heart ailments and high blood pressure. Inhibition of Angiotensin I Converting Enzyme (ACE, kinase II, EC 3.4.15.1) is currently considered to be a useful therapeutic approach in the treatment of high blood pressure. It is of great importance for controlling blood pressure by virtue of the rennin-angiotensin system. This enzyme cleaves the C-terminal of histidyl-leucine of the inactive decapeptide angiotensin I to form the octapeptide angiotensin II, a potent vaso-constrictor [239]. Although synthetic ACE inhibitors such as captopril are remarkably effective, they cause adverse side effects. Therefore, research to find safer and naturally occurring ACE inhibitors is desirable for the prevention and remedy of hypertension. ACE inhibitory peptides have been isolated from various foods as well as from enzymatic digestion of food proteins such as buckwheat, chickpea, and mushroom [240-242]. The ash gourd juice was therefore screened for ACE inhibition activity.

3.3.1.2.1. Activity guided fractionation

The ash gourd juice was fractionated with solvents of different polarity (hexane, ethyl acetate and water) and the individual fraction was screened for ACE inhibitory activity by an enzymatic assay using hippuryl-histidine-leucyl (HHL) as substrate (section 2.2.3.2). The activity was mainly observed in the aq. extract left after partitioning with hexane and ethyl acetate, while organic layers were devoid of ACE inhibitory activity. The aq extract obtained from non-irradiated control and irradiated ash gourd at a concentration of 50 mg/mL exhibited 47.28 % and 40.50 % ACE inhibition, respectively. Thus, change in the activity as a result of radiation treatment was insignificant. An ACE inhibition by 64 % was reported earlier by ash gourd pulp extract at a concentration of 10 mg/mL [243]. They demonstrated that the inhibition of ACE activity by ash gourd fruit may be due to its high phenolic contents. However, no compounds were identified by these researchers responsible for this inhibition. The lower inhibition activity observed in the present study could be due to the difference in variety and geographical origin of the vegetable studied.

The potency of an ACE inhibitor is usually expressed as IC_{50}, which is equivalent to the concentration of the compound inhibiting 50 % of ACE activity [244]. The IC_{50} value was thus determined by regression analysis of ACE inhibition (%) versus different concentrations of the extract. IC_{50} values for control and radiation processed ash gourd extracts were found to be 3.77 and 4.4 mg/mL, respectively. The aq. extract was further subjected to chromatographic separations for isolation and identification of the active principles.

3.3.1.2.2. Isolation and identification of active principles

The aqueous extract obtained after partitioning with organic solvents from non-irradiated control ash gourd was fractionated by HPLC using C18 column. Figure 56 shows HPLC chromatogram of the total aq extract of ash gourd. For preliminary screening, different regions of the HPLC chromatogram were collected and assayed for ACE inhibitory activity. As evident from table 40, maximum activity (86 %) was exhibited by fraction 1 (F1, 2 – 4 min), followed by F2 (41 %), F3 (36 %) and F4 (20 %). All others fractions collected were devoid of ACE inhibitory activity. The eluate collected corresponding to F1 was therefore subjected to re-chromatography. After re-chromatography, four major peaks (F1-1, F1-2, F1-3 and F1-4; Table 41) were collected separately to identify the active compound. The activity mainly resided in the compounds eluting between 2.75-3.00 min (F1-2). A positive reaction of this fraction with ninhydrin (development of pink/purple color) indicated its peptide nature.

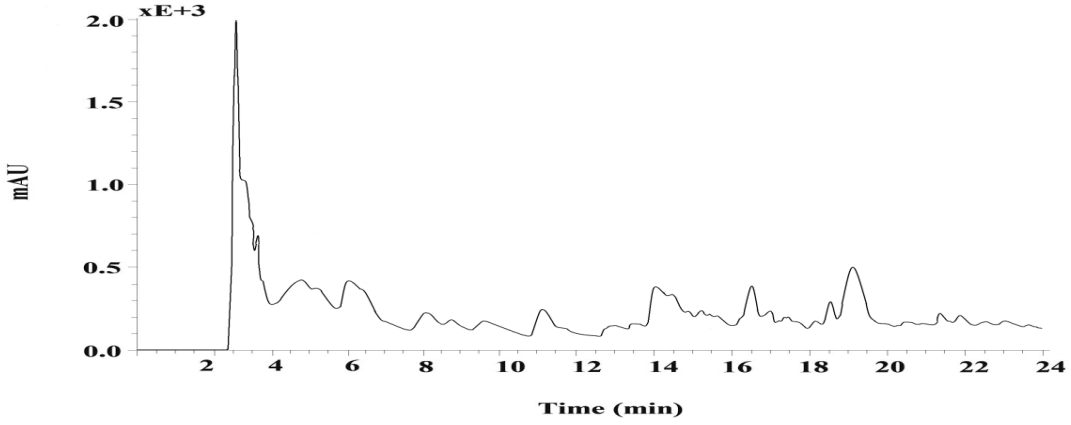


Figure 56. RP-HPLC profile of ash gourd aq extract

Table 40. The peaks collected from HPLC for preliminary screening of the fractions possessing ACE inhibition activity.

Peak No. (Fraction)	Retention time time (min)	Activity (%)
1 (F1)	2.0-4.0	86
2 (F2)	4.0-5.8	41
3 (F3)	5.8-7.8	36
4 (F4)	7.8-8.2	20
5 (F5)	8.2-9.6	-
6 (F6)	9.6-10.5	-
7 (F7)	10.5-11.5	-
8 (F8)	12.5-13.5	-
9 (F9)	13.5-14.4	-
10 (F10)	14.4-15.0	-
11 (F11)	15.0-16.0	-
12 (F12)	16.0-16.6	-
13 (F13)	16.6-18.0	-
14 (F14)	18.0-18.7	-
15 (F15)	21-23	-
16 (F16)	26-27	-

Table 41. Fractions collected from re-chromatography of region 2-4 min obtained from preliminary HPLC analysis.

Peak No. (Fraction)	Retention time	Activity (%)
1 (F1-1)	2.5-2.75	10.21
2 (F1-2)	2.75-3.00	68.25
3 (F1-3)	3.00-3.25	21.04
4 (F1-4)	3.25-4.00	5.65

The active fraction (F1-2) collected from repeated HPLC injections was pooled, evaporated, and made up in aqueous solution (10 %). The aq solution so obtained, was subsequently subjected to gel permeation chromatography (GPC), which gave rise to three major peaks (Figure 57). Each of these peaks were collected and tested for their ACE inhibitory activity. The activity mainly resided in fraction F1-2b (47 %) at a retention time of 38.9 min (Table 42).

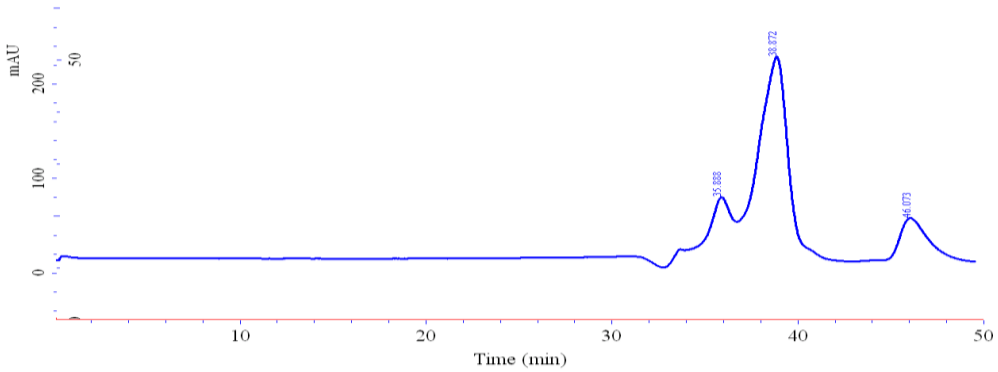


Figure 57. Chromatogram obtained from GPC of fraction 2

Table 42. Fractions collected from GPC of F1-2

Peak No. (Fraction)	Retention time (min)	Activity (%)
1 (F1-2a)	35.88	10.17
2 (F1-2b)	38.87	47.66
3 (F1-2c)	46.07	4.37

For determining the molecular weight of active fraction (F1-2b), standards of known molecular weight such as Leucine enkephaline (555.6 Da), Glu-Val-Phe (393.4 Da) and Asp-Phe (280.28 Da) were injected in GPC. The molecular weight of the active fraction (323 Da) was then calculated from the calibration curve plotted between molecular weight and V/V_0 (V - elution volume of the compound; V_0 - void volume of the column). The mass data indicated that peptide could be comprised of 2-4 amino acids. The purified fraction showed a UV absorbance at 220 and 254 nm. Absorption at 254 nm indicates presence of an aromatic amino acid in the active peptide. The active fraction (F1-2b) was subjected to mass spectral analysis using direct probe facility available in GC/MS (Shimadzu Corporation, Kyoto, Japan). Mass spectral data showed the presence of amino acids *viz*. alanine (M^+, 89) and valine (M^+, 117) in this fraction. The mass spectra of these compounds are shown in Figure 58. However, the third possible amino acid present in the peptide could not be determined in mass spectral studies. Many earlier studies on ACE inhibitory peptides including from mushroom and broccoli have reported their nature to be tripeptide [242, 245]. Cheung et al., reported the results of a series of inhibitory peptides against ACE, indicating that aromatic amino acids at the C-terminal and branched chain aliphatic amino acids at the N-terminal were suitable for a peptide binding

to ACE as a competitive inhibitor [246]. However, other reports have demonstrated that inhibitory peptides possess an aliphatic amino acid residue at their C-terminal. The results obtained in the present study, thus are consistent with the earlier reports. The confirmation of the exact sequence of the amino acids in the active peptide, however, needs further investigation.

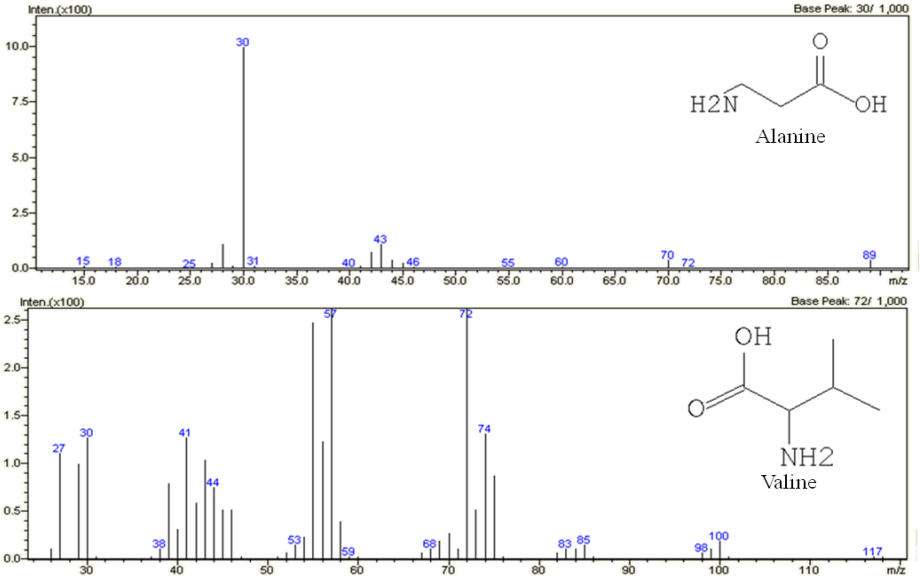


Figure 58. Mass spectra of the identified amino acids *viz.* alanine and valine in the active fraction

Thus, the consumption of ash gourd may provide significant protection against high blood pressure, and inflammation regulated by rennin-angiotensin system. This study provides evidence for pharmacological use of ash gourd in treatment of high blood pressure in traditional Indian medicine. To the best of our knowledge, this is the first report on effect of radiation processing on the ACE inhibitory activity of ash gourd. Therefore, radiation processing could be employed for improvement in the shelf life of RTC ash gourd without any substantial change in its ACE inhibition activity.

3.3.2. Cucurbitacins in pumpkin

Pumpkin belongs to Cucurbitaceae family and is known to possess several pharmacological properties such as antidiabetic, antihypertensive, antitumor, immune modulation, antibacterial, anti hypercholesterolemia, intestinal antiparasitia, and anti-inflammation [247]. The ethanolic extract of the vegetable (C*ucurbita pepo cv dayangua*) has been reported to show dose-dependent inhibitory effect on the growth of tumor cells. This anti-tumor activity, has been attributed to the presence of various cucurbitane and hexanorcucurbitane glycosides and other types of triterpenoids [248, 249]. Cucurbitane glycosides such as cucurbitacin L 2-O-β-D-glucopyranoside, cucurbitacin K 2-O-β-D-glucopyranoside; hexanorcucurbitane glycosides: 2,16-dihydroxy-22,23,24,25,26,27-hexanorcucurbit-5-en-11,20-dione 2-O-β-D-glucopyranoside and 16-hydroxy-22,23,24,25,26,27-hexanorcucurbit-5-en-11,20-dione 3-O-α-L-rhamnopyranosyl-(1 --> 2)-β-D-glucopyranoside have been isolated and identified in *cucurbita pepo* cv *dayangua* [250]. Presence of cucurbitane triterpenoids with a purine unit and cucurbitacin C & E has also been documented in pumpkin [248, 251]. Being associated with various pharmacological activities and wide occurrence, it was of interest to explore the occurrence of these bioactive constituents in pumpkin in the present study and determine the effect of radiation processing thereon.

3.3.2.1. TLC analysis

The 1% methanolic extract (as obtained in section 2.2.3.5.) of control and radiation processed samples stored for different storage periods (0, 7, 14 and 21 d) were subjected to TLC analysis. Two major bands at Rf values 0.21 and 0.32 (Figure 59) and a minor

band at Rf 0.6 was observed when the plate was stained with iodine vapors. Low Rf values of the two major bands suggested a polar nature of these compounds. In order to identify the nature of the compounds in these bands, the plate was sprayed with Liebermann-Burchard reagent (5ml acetic anhydride + 5ml conc. H_2SO_4 + 50 mL ethanol). Development of pink colored spots when the plate was heated at 120˚C, indicated triterpenoid nature of these compounds (Figure 59A). The total extract was subjected to enzymatic hydrolysis by pectinase and further subjected to TLC analysis with the same solvent system as above. Except for a spot at Rf 0.88, no spots corresponding to bands 1, 2 and 3 could be observed. This indicated a possible glycosidic nature of the compounds present in these bands. Increased activity of hydrolytic enzymes such as glycosidase and pectinase during storage in fruits and vegetables has been reported. Hence, it was of interest to determine the stability of the above compounds during radiation processing and storage. TLC analysis showed a decrease in area of the major spots (Rf 0.21 and 0.32) with storage in both control and radiation treated samples (Table 43). The observed decrease could thus be due to the activity of some of the above enzymes during storage. However, this decrease was significant only beyond 14th day of storage. No significant radiation specific effect was observed on the above compounds.

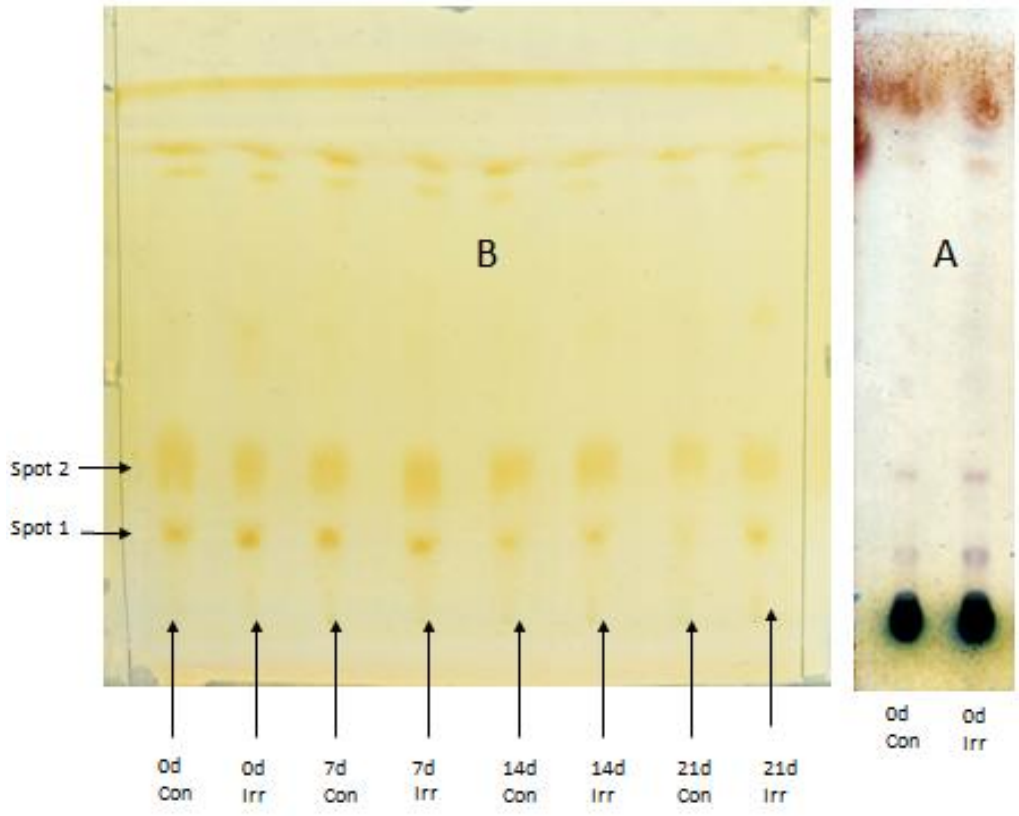


Figure 59. TLC separation of methanolic extracts obtained from control and irradiated ash gourd samples stored for various storage periods (A) sprayed with LB spray (B) stained with iodine vapors

Table 43. Peak area (sq mm) distribution of TLC spots I and 2 as measured by TLC-densitometry

	Con 0d	Irr 0d	Con 7d	Irr 7d	Con 14d	Irr 14d	Con 21d	Irr 21d
Spot 1	1757.29^{a}	1741.90^{a}	1435.38^{ab}	1473.38^{ab}	946.21^{b}	850.26^{b}	369.03^{c}	354.18^{c}
Spot 2	659.59^{a}	755.91^{a}	675.03^{a}	581.77^{ab}	196.90^{c}	174.44^{c}	25.00^{d}	27.17^{d}

Different superscripts in a row indicates significant differences between values at $p \leq 0.05$

3.3.2.2. Identification of cucurbitacins

The methanol extract and its hydrolyzate was subjected to TLC analysis using two different solvent systems (section 2.2.3.5.). (Figure 60)

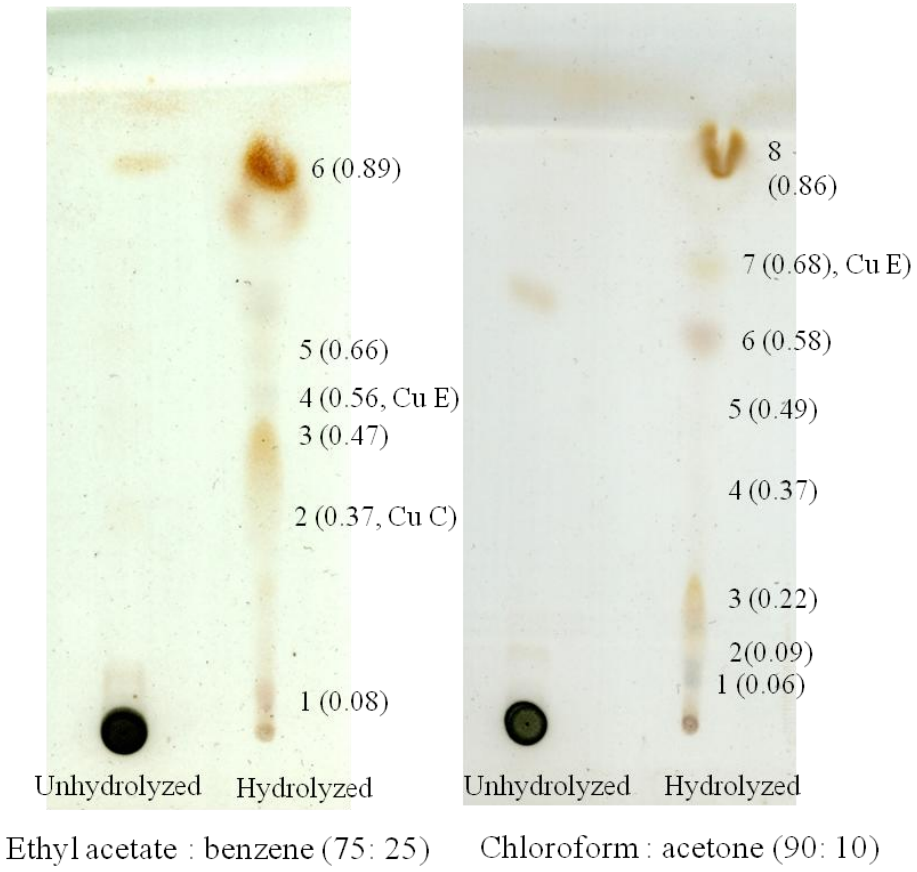


Figure 60. TLC profiles of unhydrolyzed (spot 1) and hydrolyzed (spot 2) extracts with different solvent systems (a) Ethyl acetate : benzene (75:25) (b) Chloroform : acetone (90:10); Cu C – cucurbitacin C; Cu E- cucurbitacin E.

Figure 60 depicts the TLC profile of hydrolyzed and unhydrolyzed methanol extract in two different solvent systems. The spots corresponding to cucurbitacins (Cu) were identified by comparing their Rf values with that reported in literature. While the spot at

Rf value of 0.56 (solvent system 1) and 0.68 (solvent system 2) matched with that of Cu E, that at Rf 0.37 (solvent system 1) was in agreement with Rf value of standard Cu C [252, 253]. In order to further confirm their identity, the methanol extract was subjected to HPLC analysis.

Figure 61 depicts the HPLC separation of the constituents present in the total extract. The retention time of the two cucurbitacins namely Cu C and Cu E tentatively identified above was compared with the reported literature value for these compounds. While the peak at Rt 4.27 min matched with that of Cu E glucoside, no peaks with Rt comparable to Cu C could be detected in the HPLC profile [254]. In order to further ascertain the presence of these two compounds, the hydrolyzate of the total extract was subjected to GC/MS (Figure 62) analysis.

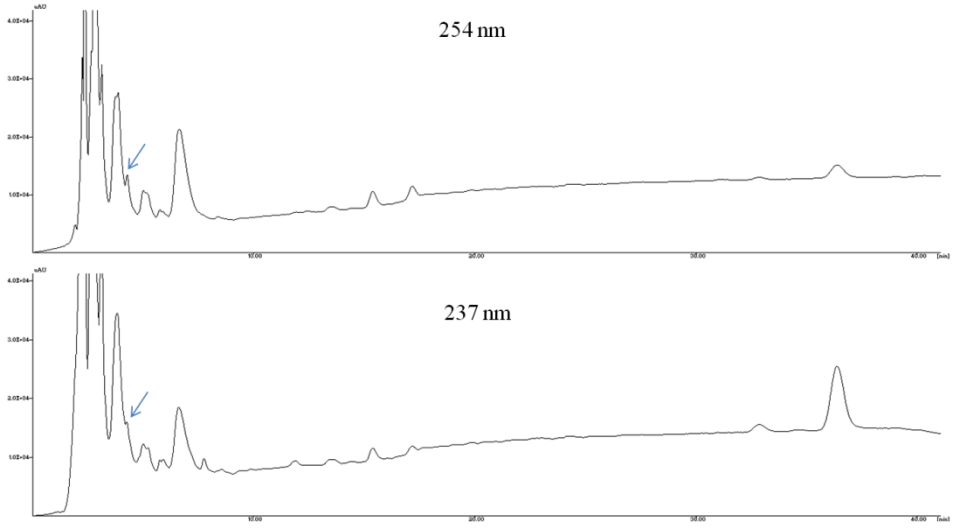


Figure 61. HPLC chromatograms obtained at 254 and 237 nm for identification of cucurbitacins. (The peak corresponding to Cu E glucoside is marked with arrow).

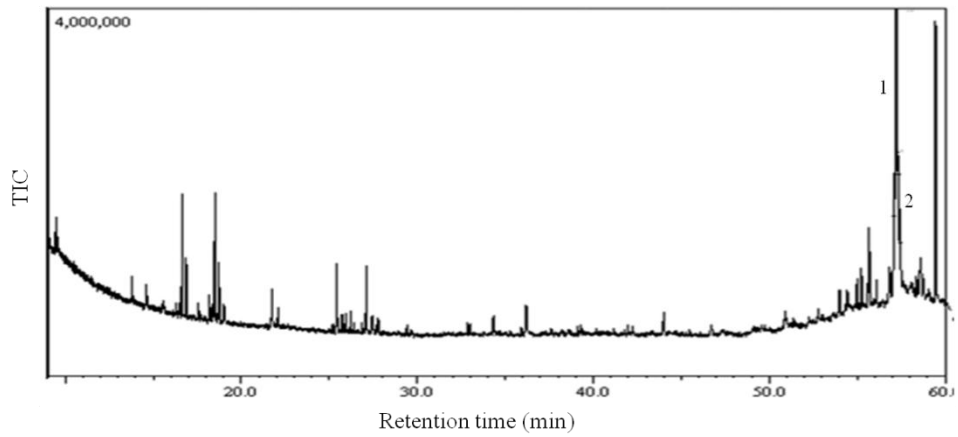


Figure 62. GC/MS profile obtained for hydrolyzate of the total extract.

The mass fragmentation (Table 44) of peak 1 and that of peak 2 (Figure 62) was in good agreement with that of the mass spectra of standard Cu C and CuE respectively , reported in literature.

The mass spectrum of peak 1 showed a fragment at m/z = 500 (M^+ - 60), corresponding to $C_{30}H_{44}O_6$. Other fragments at m/z 482 ($C_{29}H_{38}O_6$) and 470 ($C_{28}H_{38}O_6$) also matched with the fragments reported for Cu C. A very intense and characteristic peak, often the base peak was found at m/z = 43 (CH_3CO). The mass fragments at m/z = 113 ($C_6H_8O_2$) and m/z = 96 (C_6H_8O) further indicated the presence of Cu C in the hydrolyzate (Table 44).

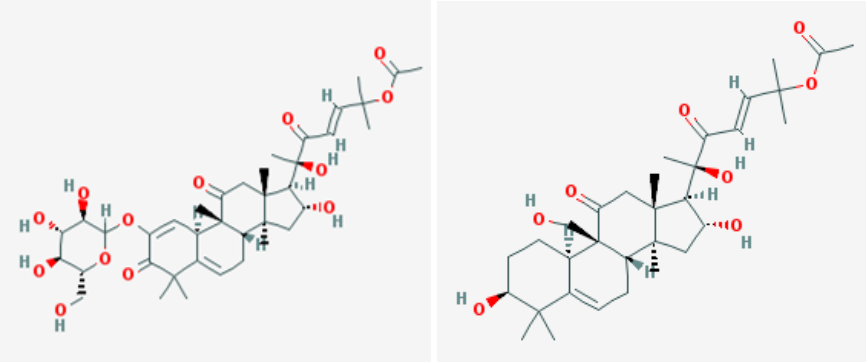
On the other hand, mass fragments of peak 2 in Figure 62 matched with that of Cu E (11 AT). The mass spectrum showed a fragment at m/z = 556 (M^+) corresponding to $C_{32}H_{44}O_8$. A very intense and characteristic peak, often the base peak was found at 96 (C_6H_8O). The mass peak at m/z = 113 might correspond to the formula $C_6H_9O_2$.

Thus, the aforementioned spectral data and Rf values on TLC (Table 44) indicated the presence of Cu C and Cu E in the hydrolyzate of total extract. The results obtained are summarized as below (Table 44)

Table 44. Rf values, maximum UV absorption and the mass fragments of Cu C and Cu E.

	Rf values		Maximum UV absorption (nm)	Mass fragments (m/z)
Identified Cucurbitacins	Ethyl acetate: Benzene (75:25)	Chloroform : acetone (90:10)		
Cu C	0.37	ND	254	500, 482, 470,113, 111, 96, 95, 69, 55, 45, 43, 41
Cu E	0.56	0.68	249.5	556, 164, 121, 113, 111, 96

Thus, based on the TLC, HPLC and GC/MS analysis of total and enzymatically hydrolyzed extract, the major triterpenes were partially identified as Cucurbitacin E glucoside and Cucurbitacin C (Figure 63). Most of the members of Cucurbitaceae have been reported to contain less than 0.01 % cucurbitacins [255]. Thus, the structure of the compounds require further verification, which is in progress.



Cucurbitacin E glucoside Cucurbitacin C

Figure 63. Chemical structure of the compounds identified in this study

3.3.3. Glucosinolates in drumstick

Drumstick (*M. oleifera*) is an important food commodity which has received enormous attention as the 'natural nutrition of the tropics'. Almost all the parts of this plant, viz-root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastro-intestinal, hematological and hepatorenal disorders [108, 111, 117]. The medicinal properties of the plant have been attributed to the presence of bioactive phytochemicals including phenolic compounds, and a unique group of compounds called glucosinolates (GSLs) and isothiocyanates (ITCs) (58, 113].

Glucosinolates are very stable water-soluble bioactive compounds. Myrosinase (EC 3.2.3.1), a class of enzymes that catalyses the hydrolysis of GSLs, is physically separated from each other in intact plant cells. Food processing methods such as physical disruption of plants by chewing, chopping and freezing results in the interaction of GSLs with myrosinase resulting in the hydrolysis of the latter and liberation of various breakdown products (isothiocyanates, nitriles, oxazolidinethiones, thiocyanate and epithionitriles) (Figure 5 in introduction). Under carefully controlled conditions designed to extract glucosinolates and isothiocyanates completely, while preventing myrosinase activity, some fresh plants have been shown to contain almost exclusively glucosinolates [60]. In contrast to intact GSL, hydrolysis products have been of interest for their diverse biological activities, ranging from antimicrobial, antioxidant and anticancer actions [58]. Among breakdown products, isothiocyanates (ITCs) are reported to have highest

biological activity. They have been reported to possess broad-spectrum antimicrobial activity against bacterial & fungal pathogens, insects and possess potent anticarcinogenic activity [256, 257]. Bioavailability of these compounds are influenced by storage and culinary processing **[258]**. Therefore, the effect of radiation treatment (1 kGy) on bioactive glucosinolates and isothiocyanates in drumstick was investigated.

3.3.3.1. Isolation and identification of glucosinolates and isothiocyanates

The glucosinolate extract was subjected to HPLC using the method detailed in section 2.2.3.7. The extract was hydrolyzed using the enzymes sulfatase and myrosinase to locate the peaks corresponding to glucosinolates. Sulfatase removes the sulfo moiety, whereas myrosinase hydrolyses the sugar moiety and releases free aglycones from the glucosinolates. The corresponding HPLC profiles are shown in Figure 64.

As evident from Figure 64, the peaks in the initial region (2 – 6 min) of the HPLC chromatogram of aq extract exhibited quantitative changes when hydrolyzed by myrosinase and sulfatase. The compounds corresponding to the major peaks in this region were thus collected for further analysis (Table 45).

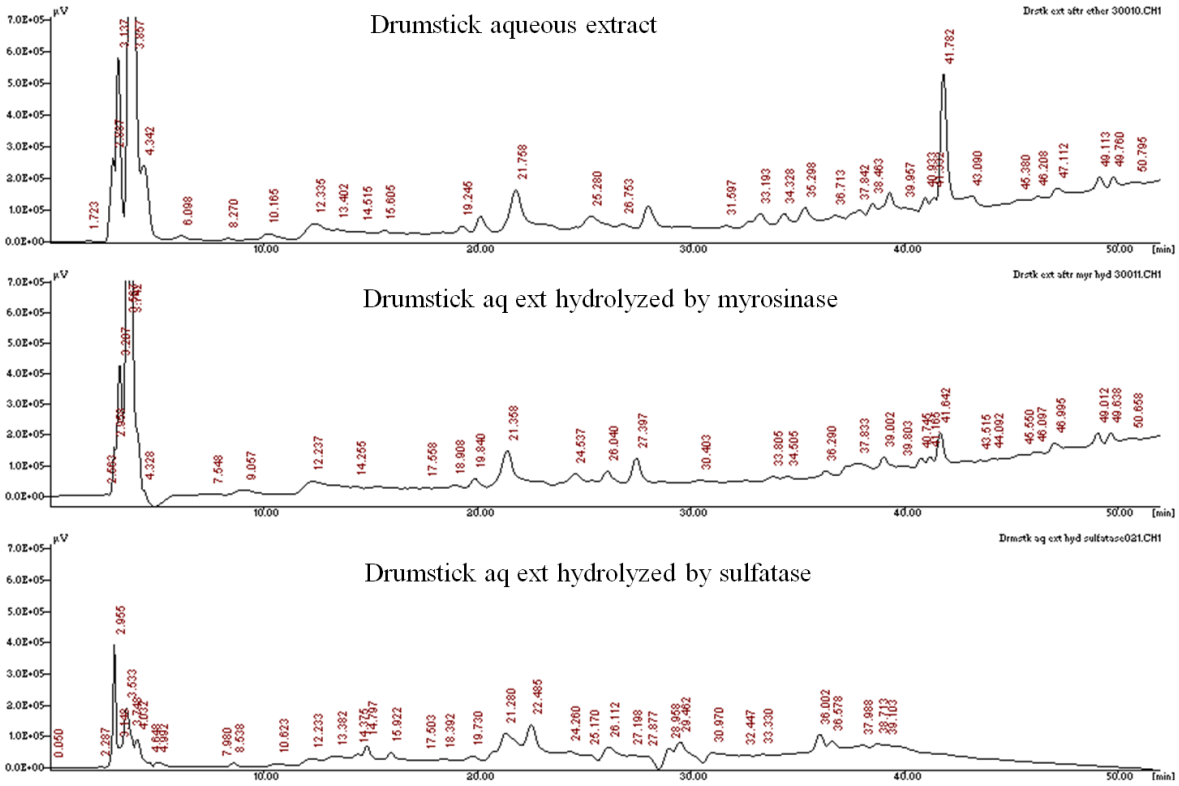


Figure 64. HPLC chromatograms obtained for drumstick aqueous extract and after hydrolysis with myrosinase and sulfatase enzyme.

Due to unavailability of standard glucosinolates, the total aq extract and the peaks thus collected were further subjected to enzymatic hydrolysis by myrosinase and the released aglycones then analyzed by GC/MS in order to identify the corresponding glucosinolates. Table 46 lists the major compounds detected in the peaks collected from HPLC of the aq extract. Although, many sulphur containing compounds were detected, no isothiocyanates could be detected in the collected peaks. The acidic conditions of the eluting solvent system (presence of TFA in solvent system, section 2.2.3.7.) might have resulted in the degradation of the isolated peaks resulting in production of sulfones and other acid

derivatives instead of isothiocyanates or nitriles. Production of different aglycones via alternative modes of degradation of glucosinolates under different conditions has been reported [259]. Therefore, HPLC could not be employed in the present study for investigating the vaiation in the glucosinolate content as a result of radiation processing and storage.

Table 45. Major compounds detected in the collected peaks as identified by GC/MS.

Peak No. (Rt in HPLC)	Major compounds identified
1 (2.84 min)	S-acetyl-2-methyl-3-mercapto propionic acid ester, carbamidoylsulfanylic acetic acid, dipropyl ester of sulfurous acid
2 (3.14 min)	Acetoin, ethyl acetate, dimethyl sulfone, 2,4-Dithiahexan-5-one
3 (3.86 min)	Dimethyl sulfone, 2,4-Dithiahexan-5-one, thiolacetic acid, 2,3-butanediol, cyclic sulfate
4 (4.34 min)	1,3-butanediol, dimethyl sulfone, pyrimidine-2,4(1H, 3H)-dione, 5-amino-6-nitroso
5 (6.09 min)	2,4-Dithiahexan-5-one, methyl thiomethyl thiol acetate

GC/MS analysis of the hydrolyzate of total aq extract resulted in identification of isopropyl isothiocyanate, isobutyl isothiocyanate and 2-butyl isothiocyanate as the major ITCs. GC/MS profile of hydrolyzed aq extract of drumstick is depicted in Figure 65. Figure 66 represents the chemical structure and mass spectral data of the identified ITCs. It has been proposed that presence of an ITC or a desulfo-GSL is proof of the parent GSL [78]. Thus, presence of isopropyl glucosinolate, 2-butyl glucosinolate and isobutyl glucosinolate in drumstick could be inferred.

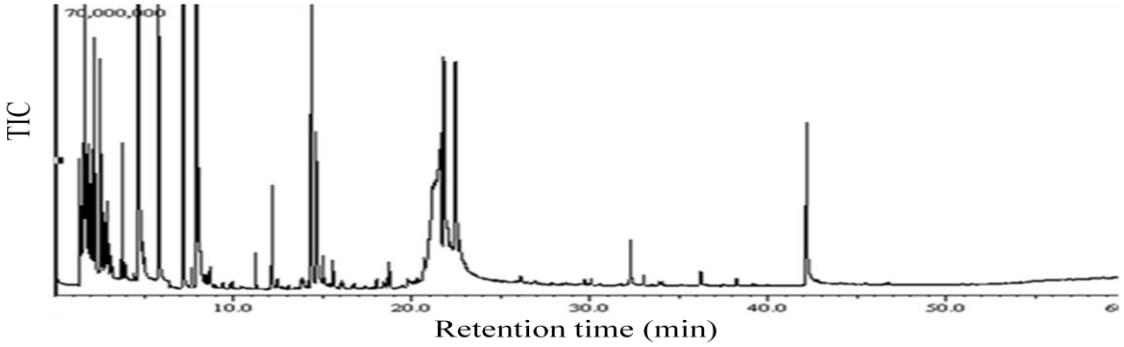


Figure 65: GC/MS profile of hydrolyzed aqueous extract of drumstick

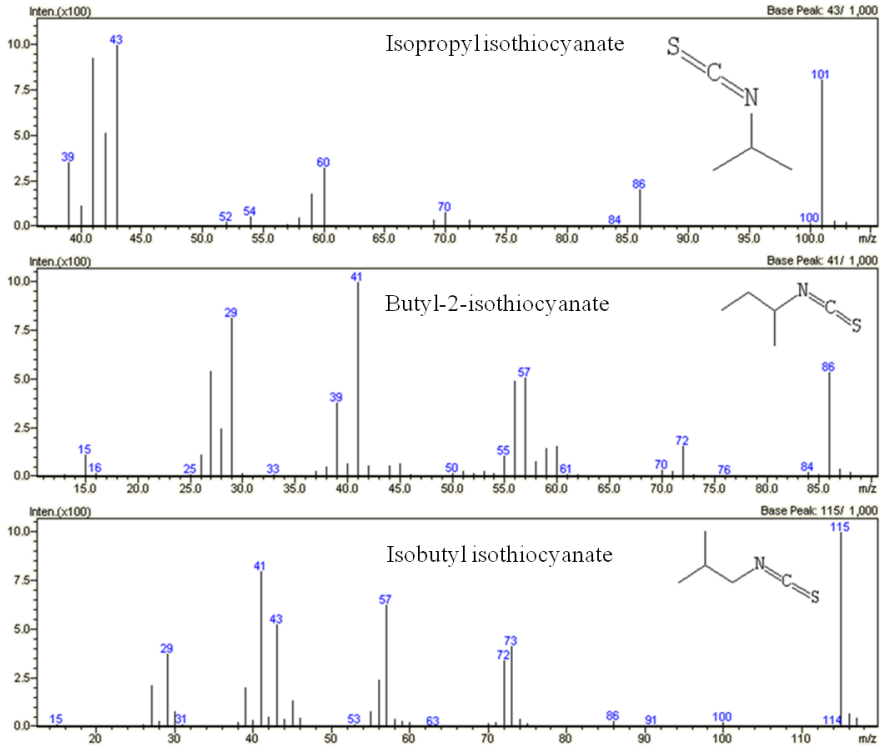
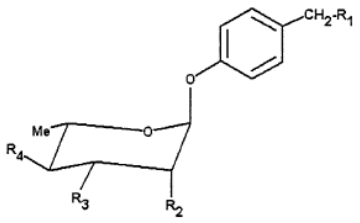


Figure 66. Chemical structure and mass spectra of isopropyl, 2-butyl and isobutyl ITC.

Several researchers have indirectly identified *Moringa* glucosinolates by analyzing the free aglycones obtained after myrosinase hydrolysis using GC/MS. These were isopropyl, isobutyl, 2-methylpropyl, 4-(α-L-rhamnopyranosyloxy)-benzyl and 4'-O-acetyl-4-(α-L-

rhamnopyranosyloxy)-benzyl in *M. peregrina* and isopropyl, 2-methylpropyl, and traces of isobutyl and 4'-o-acetyl-4-(α-L-rhamnopyranosyloxy)-benzyl in *M. pterygosperma*, and 4-(α-L-rhamnopyranosyloxy)-benzyl in *M. oleifera* and *M. stenopetala* [113]. Figure 67 shows the structures of glucosinolates previously reported in *Moringa* species. Although, isopropyl and isobutyl glucosinolates have been reported earlier in different species of *Moringa*, these compounds have not been reported in *M. oleifera* (drumstick).

Isopropylglucosinolate	$(CH_3)_2$-CH_2-CH(=$NOSO_3^-K^+$)-S-Glc
2-Methylpropylglucosinolate	$(CH_3)_2$-CH-CH_2-CH(=$NOSO_3^-K^+$)-S-Glc
Isobutylglucosinolate	$(CH_3)_3$-C-CH(=$NOSO_3^-K^+$)-S-Glc



	R1	R2	R3	R4
4-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate	-CH(=$NOSO_3^-K^+$)-S-Glc	OH	OH	OH
4"-Acetyl-4-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate	-CH(=$NOSO_3^-K^+$)-S-Glc	OH	OH	O-Ac
4-(α-L-Rhamnopyranosyloxy)-benzylisothiocyanate	-NCS	OH	OH	OH
4-(α-L-Rhamnopyranosyloxy)-benzylcyanide	-CN	OH	OH	OH

Figure 67. Structures of glucosinolates previously reported in *Moringa* species (adapted from [113])

In order to study the effect of radiation processing and storage on the glucosinolates in drumstick, the aq extracts of control and irradiated RTC drumstick at each storage duration were subjected to hydrolysis by myrosinase. The released isothiocyanates were then analyzed using GC/MS. Table 46 lists the variation in the contents of major

isothiocyanates detected in control and radiation treated drumstick at different storage intervals.

Table 46. Quantitative distribution of major isothiocyanates (µg/kg) in control and radiation processed (1 kGy) RTC drumstick at different storage periods.

	Isopropyl isothiocyanate	2-Butyl isothiocyanate	Isobutyl isothiocyanate
Con 0d	4.38 ± 0.25^{a}	1.99 ± 0.35^{a}	2.35 ± 0.22^{a}
Con 5d	3.53 ± 0.32^{ab}	1.73 ± 0.25^{a}	2.13 ± 0.20^{a}
Con 8d	1.83 ± 0.25^{b}	0.98 ± 0.28^{b}	1.65 ± 0.15^{ab}
Con 12d	1.89 ± 0.15^{b}	0.65 ± 0.20^{b}	0.83 ± 0.10^{b}
1 kGy 0d	5.10 ± 0.95^{ac}	3.57 ± 0.30^{c}	3.86 ± 0.35^{c}
1 kGy 5d	4.80 ± 0.88^{a}	2.55 ± 0.25^{ac}	3.80 ± 0.35^{c}
1 kGy 8d	4.59 ± 0.75^{a}	2.19 ± 0.15^{a}	2.95 ± 0.20^{ac}
1 kGy 12d	4.13 ± 0.52^{a}	1.98 ± 0.12^{a}	2.55 ± 0.30^{a}

Data are expressed as mean ± standard deviation (n = 6). Mean values in the same column bearing same superscript shows no significant difference ($p \leq 0.05$).

As evident from Table 46, radiation processing resulted in enhanced content of isothiocyanates. An enhancement of 16, 79 and 64 % in the content of isopropyl, 2-butyl and isobutyl isothiocyanate respectively was observed in radiation processed (1 kGy) samples in comparison to non-irradiated controls on day zero. Storage resulted in a significant decrease in the content of identified ITCs (Table 46). At the end of storage period (12 d), a decrease of 56, 67 and 65 % was observed in the contents of isopropyl, 2-butyl and isobutyl isothiocyanate respectively, in control samples. However, this decrease was only of 19, 44 and 34 % for the respective ITCs in radiation treated samples at the

end of intended storage period (12 d). Similar reports of decreased ITC content during storage have been reported in Brassica vegetables [259], where in approximately 30–50% of the total loss of ITCs in shredded vegetables stored at 4-8 °C was reported. Although, storage resulted in a significant decrease in the contents of all the three ITCs, this decrease was appreciably higher in the control than the treated samples at the end of intended storage period (12 d). Since presence of ITCs has been proposed as a proof of the existence of the corresponding parent GSLs, the changes in ITCs could be correlated with the variation in their respective GSLs. The study, thus, demonstrates an increased content of isopropyl, isobutyl and 2-butyl GSLs, thereby improving the nutraceutical value of the product, as a result of radiation processing. Radiation induced enhancement in the glucosinolate content and better retention during storage has also been reported previously in minimally processed cabbage [145. Upregulation of the genes associated with GSL biosynthesis was cited as the reason for enhanced contents of GSLs in radiation processed product. To the best of our knowledge, this is the first report showing effect of radiation processing on the glucosinolate/ isothiocyanate content of *M. oleifera*.

3.3.4. Isolation, identification and quantification of phenolic constituents and carotenoids

Dietary antioxidants have been demonstrated to play a significant health protective role. Beneficial effects of antioxidants present in fruits and vegetables have been widely reported [43]. These antioxidants such as polyphenolic compounds including flavonoids eg. flavonols, flavones have gained considerable interest as they are shown to be very

effective in protecting against cardio-vascular and other degenerative diseases [260]. Thus, there is a general tendency to increase the consumption of fresh fruits and vegetables, as this can help increase the intake of essential bioactive compounds and antioxidants. Radiation processing has been reported to increase the availability of these bioactive compounds due to increased extractability and radiolytic breakdown of their glycosidic precursors. It was therefore of interest to investigate the nature and content of phenolic compounds in the selected vegetables and determine the effect of radiation processing and storage on these constituents.

The representative HPLC chromatograms of phenolic compounds in ash gourd, drumstick and pumpkin are shown in Figure 68.

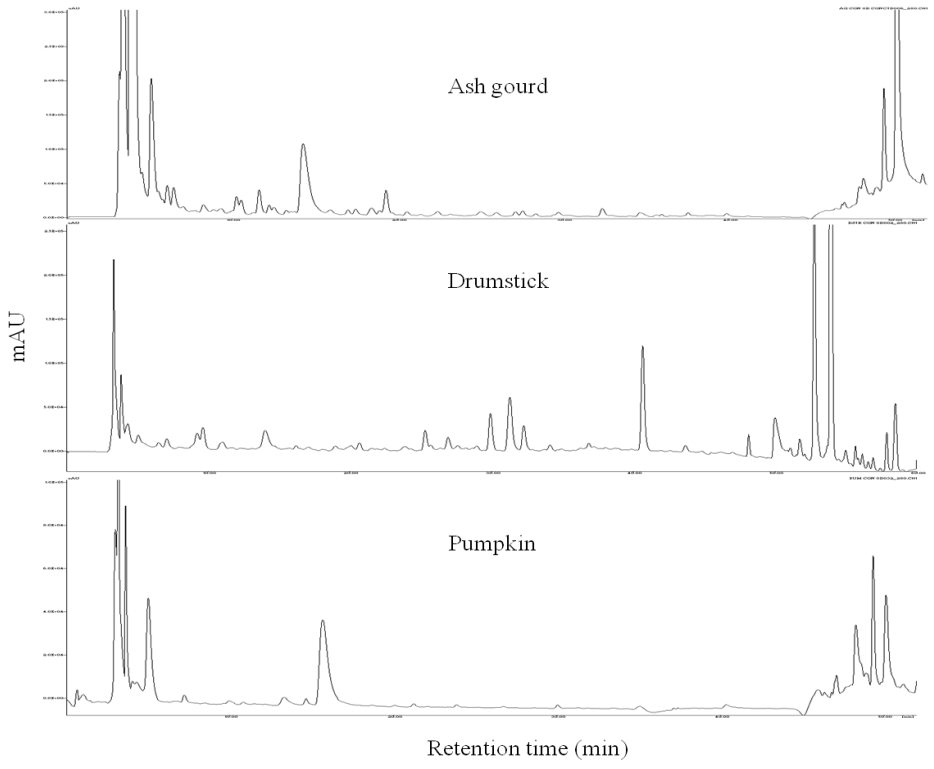


Figure 68. HPLC chromatograms obtained from ash gourd, drumstick and pumpkin at 280 nm

3.3.4.1. Ash gourd: The quantitative distribution of phenolic constituents identified in ash gourd and their changes during radiation processing and storage are represented in Table 38 (section 3.2.6.1.4.). The representative chromatogram obtained from HPLC is shown in figure 68. The major phenolic constituents identified in ash gourd included phenolic acids (α-resorcylic, *p*-hydroxy benzoic, chlorogenic, syringic, p-coumaric acid) along with catechin and naringenin (Table 38, section 3.2.6.1.4.). High antioxidant potential of ash gourd fruit has been reported earlier [261]. Different researchers have also demonstrated protective effects of ash gourd in the renal failures and injury caused by mercury chloride [100]. The antioxidant attributes of the fruit were ascribed to the presence of polyphenolics compounds including iso-vitexin, astilbin, catechin and naringenin. Besides catechin and naringenein, which were identified previously, the other phenolic acids identified in the present study have not been reported earlier. Radiation induced increase in α-resorcylic acid and a decrease in the content of chlorogenic and caffeic acid was observed. Increased α-resorcylic acid was shown in this study (section 3.2.6.1.4.) to prevent browning of irradiated ash gourd during storage. Radiation induced decrease in phenolic compounds has been reported in various plant produce [169, 170]. In general, the decrease in antioxidants and phenolic compounds is attributed to the formation of radiation-induced degradation products or the formation of free radicals. However, no significant changes in other phenolic constituents was observed as a result of radiation processing.

3.3.4.2. Drumstick

The major phenolic constituents identified in drumstick are represented in Table 47. It included phenolic acids - gallic, protocatechuic, caffeic and *o*-coumaric acid and flavonoids – catechin, epicatechin, kaempferol and rutin. Earlier reports on drumstick pods demonstrated the presence of rutin, however, phenolic acids were not reported by these workers [113]. Most of the work published on drumstick till date has been mainly focused on its leaves, seeds and flowers. Reports in literature on the nature of phenolic constituents in drumstick pods demonstrate the presence of mainly flavonoid glycosides such as quercetin-3-O-rutinoside (rutin), quercetin-3-O-glucoside with traces of kaempferol 3-O-(6”-malonyl glucoside) and isorhamnetin 3-O-(6”-malonyl glucoside). Caffeoylquinic acids (5- and 3- isomers) were detected in stem, leaves and flower with exception of roots, pods and seeds [262]. To the best of our knowledge, the presence of gallic, protocatechuic, caffeic and *o*-coumaric acids in drumstick pods is reported here for the first time. However these acids were reported earlier in drumstick seed flour by Singh et al. [263]. No significant changes in the contents of phenolic constituents identified in the present study were observed as a result of radiation processing and storage. Thus, radiation processing was found to be amenable for improving the shelf life of RTC drumstick without any significant changes in the bioactive phenolic compounds of the vegetable.

Table 47. Quantitative distribution of identified phenolic constituents (mg/kg) in drumstick

	Con 0d	Con 5d	Con 8d	Con 12d	1 kGy 0d	1 kGy 5d	1 kGy 8d	1 kGy 12d
Gallic acid	63.25 ± 5.31[a]	54.44 ± 4.97[ab]	52.17 ± 4.99[ab]	51.54 ± 4.16[ab]	62.47 ± 4.15[a]	64.37 ± 4.08[a]	60.48 ± 4.98[a]	59.14 ± 5[a]
Protocatechuic acid	51.34 ± 3.65[a]	50.11 ± 3.25[a]	47.87 ± 3.66[a]	47.36 ± 3.95[a]	51.19 ± 3.24[a]	51.12 ± 3.75[a]	49.12 ± 3.01[a]	51.05 ± 3.89[a]
Catechin	48.71 ± 3.21[a]	50.52 ± 3.15[a]	49.52 ± 2.97[a]	47.28 ± 3.67[a]	50.04 ± 3.53[a]	49.92 ± 3.08[a]	49.74 ± 3.01[a]	49.39 ± 2.91[a]
Caffeic acid	71.53 ± 3.02[a]	65.51 ± 3.07[a]	57.42 ± 3.26[ab]	61.48 ± 3.44[ab]	55.02 ± 3.63[ab]	56.66 ± 3.35[ab]	54.81 ± 2.99[ab]	51.65 ± 3.15[b]
Epicatechin	48.64 ± 4.66[a]	48.97 ± 4.66[a]	48.1 ± 5.13[a]	48.44 ± 4.09[a]	49.26 ± 5.35[a]	49.16 ± 5.1[a]	49.11 ± 4.93[a]	49.16 ± 5.27[a]
o-Coumaric acid	50.84 ± 4.05[a]	50.36 ± 3.96[a]	49 ± 4.24[a]	48.41 ± 3.53[a]	53.23 ± 3.3[a]	50.37 ± 3.3[a]	50.21 ± 2.99[a]	53.03 ± 2.81[a]
Rutin	57.19 ± 3.97[a]	49.43 ± 3.04[a]	48.87 ± 3.14[a]	49.67 ± 3.00[a]	50.58 ± 2.9[a]	48.54 ± 3.41[a]	50.9 ± 2.91[a]	50.61 ± 2.99[a]
Kaempeferol	51.67 ± 4.13[a]	47.37 ± 3.32[a]	47.33 ± 3.52[a]	43.3 ± 3.65[a]	47.92 ± 4.51[a]	47.45 ± 4.24[a]	47.43 ± 4.09[a]	47.27 ± 4.51[a]

Values are means ± SD (n = 3); Different letters indicate significant difference at $p \leq 0.05$

3.3.4.3. Pumpkin

3.3.4.3.1. Identification and quantification of phenolic constituents

The nature and content of phenolic compounds were studied in control and radiation-treated samples. Major phenolic compounds identified in the present study include

syringic, caffeic, protocatechuic, p-hydroxy benzoic, vanillic and ferulic acid along with a flavanol, quercetin (Table 48). Similar phenolic composition was earlier reported by Dragovic-Uzelac et al. [264]. Besides, chlorogenic and *p*-coumaric acid were also reported by these workers, which were not detected in the present study. A marginal but statistically significant ($p < 0.05$) decrease was noted in the contents of protocatechuic (9 %), caffeic (3 %), ferulic acid (13 %) and quercetin (7 %) due to radiation processing. No significant ($p < 0.05$) effect of radiation treatment was, however, observed on the content of all other phenolic constituents identified (Table 48).

A significant ($p < 0.05$) reduction in the content of all the phenolic compounds was observed during storage in control samples. The losses ranged from 6 % for vanillic acid to a maximum reduction of 31 % in the content of protocatechuic acid on day 21. Increased physiological and microbial spoilage during storage, resulting in enhanced degradation of sensitive compounds such as phenolic constituents could possibly explain the decreased content observed. However, radiation-treated samples had a higher content of phenolic constituents as compared to non-irradiated controls at the end of storage period of 21 d (Table 49). Better maintenance of physiological and microbial quality in the radiation-treated samples could have prevented the leaching out of phenolic compounds and their exposure to air, resulting in lesser oxidation and thus maintaining their content during storage.

Table 48. Quantitative distribution of identified phenolic constituents and carotenoids (mg/kg) in pumkin

	Con 0d	Con 7d	Con 14d	Con 21d	1 kGy 0d	1 kGy 7d	1 kGy 14d	1 kGy 21d
Protocatechuic acid	42.27 ± 2.31^{a}	39.61 ± 1.97^{ab}	32.63 ± 0.99^{cd}	29.6 ± 2.16^{d}	38.98 ± 1.15^{b}	33.57 ± 2.08^{c}	33.53 ± 0.98^{c}	32.32 ± 1^{cd}
p-hydroxy benzoic acid	32.52 ± 1.65^{a}	32.88 ± 1.25^{a}	33.82 ± 1.66^{a}	31.46 ± 1.95^{a}	31.23 ± 1.24^{a}	33.33 ± 1.75^{a}	32.04 ± 1.01^{a}	32.9 ± 0.89^{a}
Vanillic acid	30.9 ± 1.21^{c}	32.97 ± 1.15^{bc}	31.79 ± 0.97^{bc}	28.83 ± 1.67^{a}	31.72 ± 1.53^{bc}	33.25 ± 1.08^{bc}	33.58 ± 1.01^{b}	33.89 ± 0.91^{b}
Caffeic acid	36.75 ± 1.44^{ab}	34.98 ± 1.26^{bc}	31.82 ± 1.07^{d}	28.86 ± 1.02^{e}	35.39 ± 1.63^{cd}	37.62 ± 1.35^{a}	35.58 ± 0.99^{abc}	37.52 ± 1.15
Syringic acid	52.34 ± 2.66^{a}	48.03 ± 2.66^{ab}	49.63 ± 3.13^{a}	41.45 ± 2.09^{c}	49.7 ± 3.35^{a}	50.21 ± 3.1^{a}	49.76 ± 2.93^{a}	43.5 ± 3.27^{b}
Ferulic acid	38.67 ± 2.05^{a}	36.28 ± 1.96^{ab}	33.48 ± 2.24^{bcd}	34.56 ± 1.53^{bc}	33.44 ± 1.3^{d}	31.43 ± 1.3^{d}	32.28 ± 0.99^{cd}	32.53 ± 0.81^{cd}
Quercitin	41.19 ± 1.97^{a}	42.69 ± 1.04^{a}	35.47 ± 1.14^{c}	29.21 ± 1^{d}	38.14 ± 0.9^{b}	35.07 ± 1.41^{c}	34.1 ± 0.91^{c}	34.17 ± 0.99
Lutein	7.76 ± 1.26^{a}	7.62 ± 2.14^{a}	5.87 ± 1.28^{abc}	5.81 ± 1.39^{abc}	6.81 ± 2.11^{ab}	4.36 ± 1.52^{bc}	3.26 ± 0.96^{c}	3.51 ± 0.84^{c}
β-carotene	4.75 ± 1.21^{a}	4.84 ± 1.65^{a}	3.62 ± 0.96^{ab}	3.42 ± 0.65^{ab}	4.80 ± 0.96^{a}	3.06 ± 0.63^{ab}	2.13 ± 0.32^{b}	2.40 ± 0.26^{b}

Values are means ± SD (n = 3); Different letters indicate significant difference at $p \leq 0.05$

3.3.4.3.2. Identification and quantification of carotenoids

Carotenoids are the natural plant pigments responsible for the bright yellow-orange colour of pumpkin. Besides, they are known to possess significant antioxidant activity and a range of other biological activities [265]. Content of β-carotene and lutein, major carotenoids identified in the present study are shown in Table 49. The representative HPLC profile obtained for carotenoids is shown in Figure 69.

The content of β-carotene and lutein in fresh control pumpkin samples was 4.75 ± 1.21 mg/kg and 7.76 ± 1.26 mg/kg, respectively. No significant effect of radiation processing was observed on the content of both the carotenoids immediately after irradiation. Similar observations have been reported in irradiated carrots and cucumber and sliced tomatoes [87, 266]. However, a significant ($p < 0.05$) decrease was observed during storage in both control and irradiated samples (Table 11). The content of beta carotene and lutein at the end of storage (21 d) was found to be 3.42 ± 0.65 and 5.81 ± 1.39 respectively in control and 2.40 ± 0.26 and 3.51 ± 0.84 mg kg^{-1} respectively in radiation-treated samples. Similar results have been documented for various radiation-treated Indian potato cultivars by [267]. wherein they reported 50 % reduction in the total carotenoid content after 6 months of storage. Thus, from the data obtained in the present study, it could be concluded that the overall nutritional quality of the radiation-treated (1 kGy) product was better than the corresponding control at the end of the intended storage (21 d).

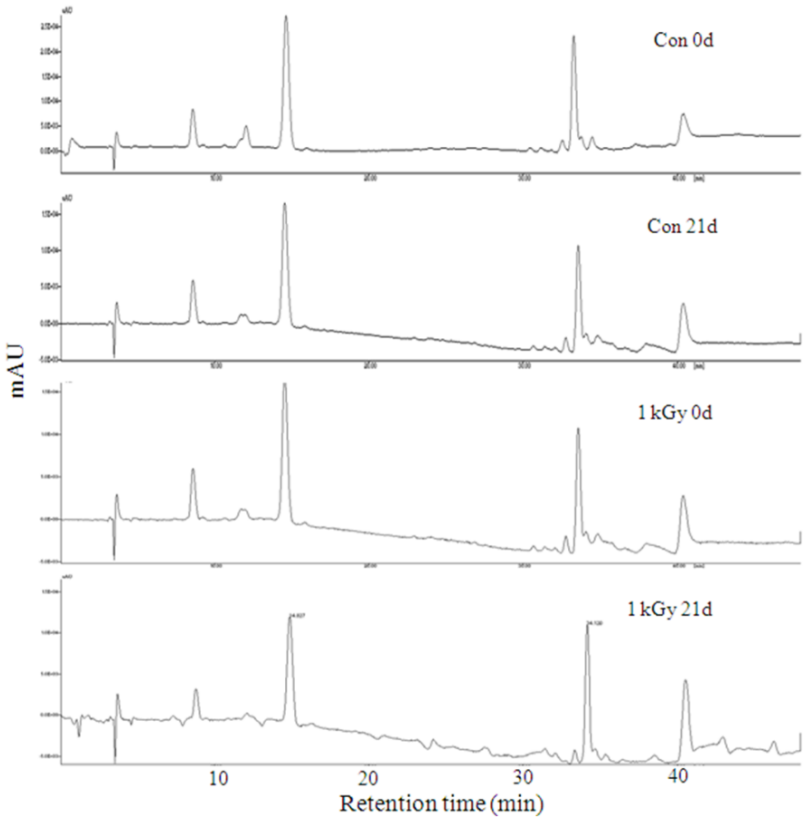


Figure 69. HPLC chromatograms obtained for control and radiation processed pumpkin (0 d and 21 d) carotenoids (450 nm).

3.4. Conclusions

The experimental design approach was successfully employed for optimization of radiation dose and storage time for obtaining RTC products of ash gourd, drumstick and pumpkin with acceptable microbial and sensory quality. Third-order polynomial equations explained the complex interactions among radiation dose, storage duration and quality attributes of the RTC products. At an optimum dose of 2 kGy, a shelf life of 12 d for ash gourd; and at a dose of 1 kGy a shelf life of 12 and 21 d for drumstick and pumpkin respectively was achieved. Radiation-treated products had better nutritional attributes as compared to the control at the end of storage period. Analysis of developed products by QDA demonstrated that the processed products had acceptable aroma, taste and textural properties. The mathematical approach for obtaining process parameters can be useful for commercial production as the processing conditions can be suitably optimized based on quality characteristics required, thus reducing severity of treatments. The study demonstrated the efficacy of gamma irradiation for extending the shelf-life of minimally processed vegetables.

Free and bound aroma compounds of the selected vegetables – ash gourd, drumstick and pumpkin were identified. SPME in case of free aroma and SPE for bound aroma were found to generate better qualitative and quantitative data in all the three vegetables. Alcohols, aldehydes, ketones and terpenes were the major classes of compounds identified. Interference with the solvent in the initial regions of chromatograms and losses of volatiles during evaporation step in SDE restricted the identification of many lower boiling aroma compounds in SDE.

GC-O analysis of the aroma extracts was successfully employed for the identification of key odorants in the vegetables studied. Acetoin, octanal and nonanal for ash gourd; benzothiazole, decanal and 2-dcenal in drumstick and a combination of 6Z-nonenal and 2E, 6Z-nonadienal in case of pumpkin were demonstrated to be the key odorants responsible for the characteristic aroma of the respective vegetable.

To the best of our knowledge, this is the first report on identification of aroma constituents in these vegetables. As bound aroma glycosides serve as a reservoir of potent aroma, the data so generated might be useful in exploiting this pool for enhancing/modifying the overall aroma quality of the vegetables by using suitable processing steps. Information about key odorants may be utilized in reconstitution of the aroma formulations, which can be utilized in the food/ flavor industry.

Radiation processing resulted in significant changes in aroma profile of all the three vegetables studied. However, the changes varied in each vegetable. Contents of alcohols increased in response to radiation processing and storage in ash gourd and pumpkin which could be attributed to the radiolytic breakdown of their corresponding glycosidic precursors.

The contents of major carbonyl compounds decreased with radiation processing and storage in ash gourd, while a significant increase in the content of hexanal and trans-2-hexenal was observed in radiation processed drumstick. The increase in aldehydes content could be attributed to radiation induced lipid radiolysis resulting in release of linolenic acid and its subsequent conversion to aldehydes via lipoxygenase (LOX) pathway. On the other hand, conversion of aldehydes to corresponding branched chain alcohols and

subsequently to esters could be the reason for decreased contents of aldehydes in ash gourd. No significant changes were observed in carbonyl compounds of pumpkin in response to irradiation.

A considerable difference in the effect of radiation processing on the content of glycosidic precursors was observed among the three vegetables studied. Two types of radiation effects were observed. Decrease in glycosidic precursors was seen in ash gourd and pumpkin, while their contents increased in irradiated drumstick. Both radiation induced increased extractability and degradation was observed. It can be concluded that both these changes can occur simultaneously and independent of each other. Despite the changes in the content of some of the aroma compounds during storage and radiation processing as noted instrumentally and statistically, they were not sufficient to be observed by the sensory panel. Hence the radiation processed product developed, had good sensory acceptability at the end of storage period.

Radiation processing inhibited browning in RTC ash gourd during storage. Enhanced electrolytic leaching due to softening of tissue structure and enhanced PAL activity were found to play a major role in increased browning of control samples during extended storage. Radiation induced increase in the content of α-resorcylic acid resulted in inhibition of ash gourd PPO that subsequently, prevented browning in radiation processed samples. The current work demonstrated the feasibility of radiation processing as an effective post harvest processing method in inhibiting surface browning in RTC ash gourd. Thus, besides being highly effective method of ensuring food safety, γ-irradiation provides an improved benefit in terms of maintaining visual quality of the product. To the

best of our knowledge, this is the first report on radiation induced browning inhibition in RTC ash gourd.

At the optimum doses employed for shelf-life extension, (2 kGy for ash gourd; 1 kGy for drumstick and pumpkin), the bioactive principles were not affected significantly. The plant growth regulating and ACE inhibition capacity of ash gourd was not altered by radiation treatment. The contents of major isothiocyanates identified in drumstick (isopropyl isothiocyanate, isobutyl isothiocyanate and 2-butyl isothiocyanate) was 2-3 times higher in radiation treated drumstick as compared to non-irradiated control at the end of storage period (12 d), thus resulting in improved nutraceutical value of the vegetable. An increased content of α-resorcylic acid in irradiated ash gourd resulted in PPO inhibition and consequently browning development on the cut surfaces of the vegetable. A marginal decrease in the phenolic contents was observed in radiation-treated pumpkin, whereas in drumstick, they were unaffected. Thus radiation processing was found to be amenable for extending the shelf life of RTC products of ash gourd, drumstick and pumpkin without any adverse effect on the bioactive principles. To the best of our knowledge, this study demonstrates for the first time the effect of radiation processing on shelf life improvement and various phyto-chemical aspects of RTC ash gourd, drumstick and pumpkin.

CHAPTER 4

SUMMARY

The present study aimed at determining the feasibility of radiation processing for improving the shelf life of ready-to cook (RTC) vegetables of Indian origin. It was also of interest to determine the changes, if any, in the quality of the processed products as a result of radiation processing. The vegetables selected for the study were: ash gourd, drumstick and pumpkin. The main attributes studied included microbial quality, color, texture, sensory acceptability and nutritional quality. Changes in bioactive compounds and their precursors during such a treatment were also investigated.

The highlights of the study are summarized below:

- Radiation processing of RTC ash gourd, drumstick and pumpkin, packed in polystyrene trays and wrapped in cling films was demonstrated to improve their shelf-life when stored at 10 °C
- The full factorial experimental design approach was successfully employed for optimization of radiation dose and storage time for obtaining RTC products of ash gourd, drumstick and pumpkin with acceptable microbial and sensory quality.
- At an optimum dose of 2 kGy a shelf life of 12 d for ash gourd; and at a dose of 1 kGy a shelf life of 12 and 21 d for drumstick and pumpkin respectively was achieved.
- Application of gamma radiation at optimum doses resulted in development of RTC products with desired microbial and sensory quality.
- Radiation-treated products had better nutritional quality (antioxidant potential, vitamin C, total phenolic & flavonoid content) as compared to the non-irradiated controls at the end of intended storage period.

- The use of radiation processing in combination with cling wrap packaging was shown to establish aerobic conditions within the package, thus inhibiting the growth of anaerobic pathogens.
- The mathematical approach used for obtaining process parameters was demonstrated to be useful for commercial production as the processing conditions can be suitably optimized based on quality characteristics required thereby reducing severity of treatments.
- Gamma irradiation inhibited surface browning in RTC ash gourd during storage. A decreased quinone formation and enzyme (PAL and PPO) activities and an increased α-resorcylic acid content, a known PPO inhibitor, as a result of radiation processing was demonstrated to be responsible for the observed inhibition in browning.
- The nature and contents of free and glycosidically bound aroma compounds was investigated in all the three vegetables. Acetoin, octanal and nonanal in ash gourd; benzothiazole, decanal and 2E-decenal in drumstick and a combination of 6Z-nonenal and 2E, 6Z-nonadienal in case of pumpkin were identified as the key odorants by employing GC-O technique.
- The effect of radiation processing and storage on free and bound aroma constituents was studied using principal component analysis (PCA).
- Radiation induced enhancement in the contents of most of the alcohols was observed in ash gourd and pumpkin. This could be attributed to the radiolytic breakdown of their corresponding glycosidic precursors. An increase in the

content of hexanal and trans-2-hexenal was also observed in radiation processed drumstick.

- Despite the changes in the content of some of the aroma compounds during storage and radiation processing as noted instrumentally and statistically, these changes were not sufficient to bring about alteration in the sensory quality. The radiation processed products developed, had good sensory acceptability at the end of storage period.
- Acetoin glucoside, a major aroma compound in ash gourd, as a plant growth regulator was demonstrated. Molecular mechanism of this growth promotion was shown to be due to the upregulation of RanB1 protein (an important regulatory protein) in tobacco leaf discs treated with acetoin glucoside.
- Angiotensin converting enzyme (ACE) inhibitory activity was demonstrated in ash gourd juice. The compound responsible for the activity was found to be a small peptide (323 Da), comprising of alanine and valine. The ACE inhibition activity of the vegetable juice remained unaffected in response to radiation treatment.
- Radiation processing did not cause any significant changes in the content of cucurbitacin C & cucurbitacin E glucoside, the major triterpenes of pumpkin.
- Presence of isopropyl glucosinolate, 2-butyl glucosinolate and isobutyl glucosinolate in *Moringa oleifera* was shown for the first time. Radiation induced enhancement in the contents of these compounds at the end of storage (12 d) was demonstrated.

- No significant effect of radiation treatment was observed on the major phenolic constituents identified in the selected vegetables.
- Radiation processing was found to be amenable for extending the shelf life of RTC products of ash gourd, drumstick and pumpkin without any adverse effect on the bioactive principles.
- To the best of our knowledge, this study demonstrates for the first time the effect of radiation processing on shelf life improvement and various phyto-chemical aspects of RTC ash gourd, drumstick and pumpkin.

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List of Publications arising from the thesis

Journal

a. Published:

1. **Sharma, J.,** Chatterjee, S., Kumar, V., Variyar, P. S., & Sharma, A. (2010). Analysis of free and glycosidically bound compounds of ash gourd (*Benincasa hispida*): Identification of key odorants. Food Chemistry, *122*(4), 1327-1332.

2. **Tripathi, J.,** Chatterjee, S., Vaishnav, J., Variyar, P. S., & Sharma, A. (2013). Gamma irradiation increases storability and shelf life of minimally processed ready-to-cook (RTC) ash gourd (*Benincasa hispida*) cubes. Postharvest Biology and Technology, 76, 17-25.

3. **Tripathi, J.**, Gupta, S., Mishra, P. K., Variyar, P. S., & Sharma, A. (2014). Optimization of radiation dose and quality parameters for development of ready-to-cook (RTC) pumpkin cubes using a statistical approach. Innovative Food Science & Emerging Technologies, 26, 248-256.

b. Manuscript under preparation

1. Tripathi, J., Adiani, V., Ghosh, S. B., Ganapathi, T. R., Bauri, A.K., Chatterjee, S., Kulkarni, V.M., Variyar, P. S., & Sharma, A. Identification of GTP binding nuclear protein Ran as an upregulation target in acetoin glucoside mediated plant growth enhancement

2. Tripathi, J., & Variyar, P. S. Optimization of radiation dose and quality parameters for development of ready-to-cook (RTC) drumstick using a statistical approach.

3. Tripathi, J. & Variyar, P. S. Mechanism of browning inhibition in radiation processed ready-to-cook ash gourd.

4. Tripathi, J., & Variyar, P. S. Analysis of free and glycosidically bound compounds of drumstick (*Moringa oleifera*) and pumpkin (*Cucurbita pepo*): Identification of key odorants.

Conferences

1. Sharma, J., Vivekanand Kumar, Chatterjee,S., Variyar,P. S. and Sharma, A. Identification of aroma impact compounds of ash gourd: Proceedings of the 13th ISMAS-WS on Mass Spectrometry,(2008),184-190,ISBN:978-81-904442-1-7.
2. Sharma, J., Chatterjee, S., Gupta, S., Kumar, V., Variyar, P. S. and Sharm, A. Development of shelf stable radiation processed ready-To-Cook (RTC) Indian vegetables. Peaceful Uses of Atomic Energy, 29th Sep-1st Oct. 2009 held at Vigyan bhawan, New Delhi.
3. Tripathi, J., Variyar, P. S. and Sharma, A. Mechanism of browning inhibition in radiation processed ready-to-cook ash gourd. Poster presented at DAE-BRNS Life Science Symposium (LSS) 2012, "Trends in Plant, Agriculture and Food Science" (TIPAFS) at multipurpose hall, Training School Hostel, Anushakti Nagar, Mumbai, Dec, 17-19 2012.
4. Tripathi, J., Gupta, S., Variyar, P. S. and Sharma, A. Improving shelf life of gamma radiation processed Ready-to-cook (RTC) pumpkin cubes: A multivariate statistical

approach. Poster presented at IFCON-2013, 18-21 December, 2013 held at CSIR-CFTRI, Mysore.

5. Tripathi, J., Gupta, S., Variyar, P. S. and Sharma, A. Improving shelf life of gamma radiation processed Ready-to-cook (RTC) drumstick: A multivariate statistical approach. Poster presented at LSS-2015, 3-5 Feb, 2015 held at NUB, Anushakti Nagar, Mumbai.

Others

1. Sharma, J., Chatterjee, S., Gupta, S., Kumar, V., Variyar, P. S. and Sharma, A. Development of shelf-stable radiation processed Ready-to-cook (RTC) Indian vegetables. Thematic volume evolved from the proceedings of International Conference on Peaceful uses of atomic energy-2009, Sep 29- Oct 1, 2009, Vigyan Bhavan, New Delhi, (2011), 154-158.
2. Tripathi, J., Gupta, S., Kumar, V., Chatterjee, S., Variyar, P. S and Sharma, A. Processing food for Convenience: Challenges and Potentials. BARC Newsletter, (2011), 322, 55-60.

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