SCREENING SOMACLONES OF GINGER (*Zingiber officinale* ROSC.) FOR VALUE ADDITION

By

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THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Screening somaclones of ginger (*Zingiber officinale* Rosc.) for value addition"** is a bonafide record of research work done by me during the course of research and that it has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Screening somaclones of ginger (*Zingiber officinale* Rosc.) for value addition"** is a bonafide record of research work done independently by **Mr. Anand Kankanawadi (2013 - 12 - 117)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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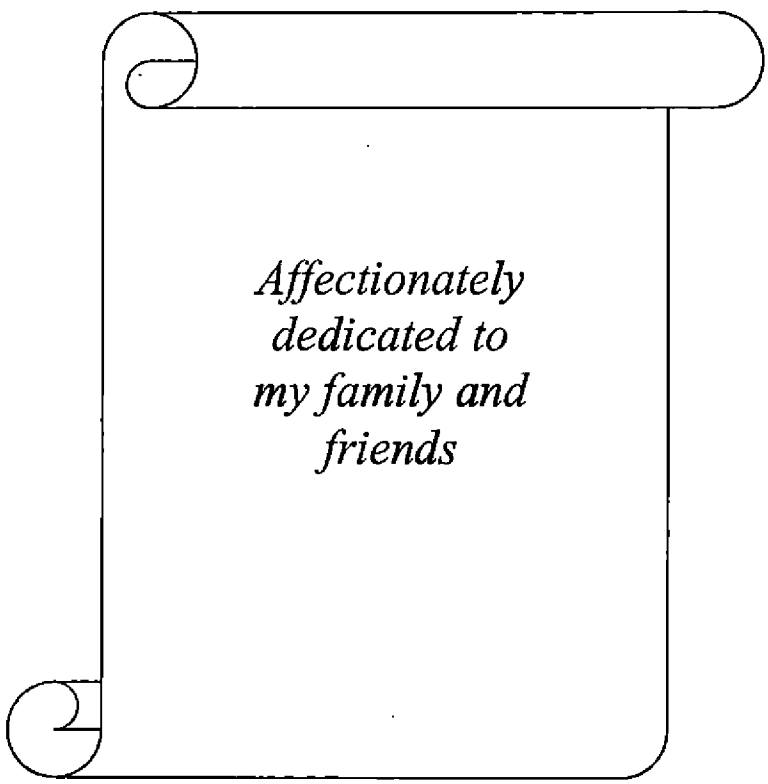
Affectionately
dedicated to
my family and
friends

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Introduction

INTRODUCTION

Ginger is one of the oldest, widely cultivated and used spice around the globe. Rhizome is the economic part used either in the fresh or dried form. It forms a part of the traditional medical practices of all the ginger growing countries. It is also a common food additive in a number of foods and beverages, due to its characteristic pleasant aroma and pungency. In Western countries, ginger is used in ginger bread, biscuits, cakes, puddings, soups, pickles, beer and wine. In Saudi Arabia, it is predominantly used for flavouring coffee. The essential oil and oleoresin from ginger are valuable products responsible for the characteristic flavour and pungency. Essential oil and oleoresin contents of ginger rhizome varies from 1-3% and 4 -7.5% respectively. The active components of ginger are reported to be α-zingiberene, 6-gingerol, β-sesquiphellandrene, 6-shogaol, α-farnesene, β-bisabolene and α-curcumene (Zhan *et al.,* 2008). Gingerols, the pungent principle of ginger are biologically the most active components that make significant contribution towards the medicinal applications of ginger as anti-inflammatory, cardiotonic and cancer chemopreventive (Kizhakkayil and Sasikumar, 2011).

Ginger is mainly grown in Jamaica, India, Sierra Leone, Nigeria, South China, Japan, Taiwan and Australia. India is the largest producer, consumer and exporter of ginger in world, with an area of 133 thousand ha and 655 metric tonnes production (2014-2015). The export during 2012-2013 was 19, 850 tonnes valued at Rs. 16, 863, 10 lakhs. In India Kerala, Karnataka, Orissa, Assam, Meghalaya, Arunachal Pradesh, Gujarat, Sikkim, Mizoram, West Bengal, Andhra Pradesh are the major producers. Kerala has a prominent position as a ginger growing State and produces Cochin and Calicut ginger, renowned for their intrinsic qualities. In spite of these, significant strides could not be made in the processing sector unlike small growers like Australia and Fiji, which are major exporters of value added products from ginger.

Dry ginger continues to be the only primary product produced and marketed from Kerala resulting in cyclic price crash and farmer's suicide. Among spices, ginger is most suitable for product diversification. The nutraceutical and pharmaceutical significance of ginger offer great scope for diversifying the product range and a variety of ginger products such as extracts, preserve, candy, beverages, paste, syrup, bread, biscuits, wine and soft drinks are available in the market. Considering the pharmaceutical significance of gingerol and related pungent compounds, products with standardized contents are necessary to catch up with international trends. Ginger 'generally recognized as safe' by the Food and Drug Administration (FDA) of the United States and has gained considerable attention as a botanical dietary supplement in developed countries, opening ample export potential. The refreshing pleasant aroma, biting taste and carminative property of ginger make it an indispensable ingredient of food processing industry.

Fibre, volatile oil and pungency level are the most important criteria in assessing suitability of ginger rhizomes for particular processing purposes. These quality components vary in fresh and dried forms of ginger and also at different maturity stages.

Elite varieties satisfying the requirements for specific end products are the need of the hour to capitalize on the processing front. Being obligatory asexual and propagated exclusively through vegetative means, the variability available is limited. Therefore, works initiated to induce variability through polyploidy, at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, has succeeded in the development of two autotetraploids (Sheeba, 1996). In order to increase the spectrum of variability, induction of variation *in vitro* through indirect methods of regeneration and mutagenesis in two induced polyploids and diploid cultivar HP was attempted as part of DBT funded project from 2006 to 2010. This has resulted in development of potential variants which on primary evaluation revealed wide

variability in morphology and yield attributes (Kurian, 2010). Detailed evaluation of a set of somaclones by Dev (2013) further reported variability in morphology, yield, quality and tolerance to rhizome rot and bacterial wilt diseases.

The present investigation was carried out with the following objectives

1) To evaluate ginger somaclones for variations in quality attributes and to identify novel chemotypes through chemoprofiling
2) To screen ginger somaclones for product diversification and to identify elite types for value added products.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The investigations on "Screening somaclones of ginger (*Zingiber officinale* Rosc.) for value addition" focus on evaluating 40 somaclones for variations in quality attributes and identify novel chemotypes through chemoprofiling and screening for product diversification and identify elite types for value added products. The literature related to these aspects in crop plants with special reference to ginger are dealt in this chapter.

2.1 CLONAL VARIATIONS IN GINGER

2.1.1. Rhizome yield

Muralidharan (1973) reported that cultivar Rio-de-Janeiro was the highest fresh ginger yielding variety, whereas the dry ginger yield was lowest in the cultivar. Dry ginger yield was highest in cultivar Tura. Cultivars Moran, Nadia and Thingui are the other high yielders and were more or less on par with cultivar Rio-de-Janeiro.

According to Nybe *et al.* (1982), Nadia was the highest fresh rhizome yielding cultivar followed by Maran, Bajpai, and Narasapattam. Cultivar Nadia also gave the higest yield of dry ginger. Sreekumar *et al.* (1982) found that cultivar Rio-de-Janeiro and Kuruppumpadi were the best yielders.

Ramachandran (1982) and Ramachandran and Nair (1992) reported successful production of stable tetraploid lines in cvs. Maran and Mananthody. The polyploids were more vigorous than the diploids and flowered during the second year of induction. The stable tetraploid lines had larger, plumpy rhizomes and high yield (198.7 g/ plant). However, the essential oil content was lower (2.3%) than the original diploid cultivar. There was a considerable increase in pollen fertility in tetraploids.

Rai *et al.* (1999) recorded the highest fresh rhizome yield (462.6 g/ plant) in cv. 'Gorubathaney' and the lowest (234.5 g/plant) in 'Suprabha'.

Ram and Sheo (1999) evaluated twenty-one indigenous and exotic genotypes of ginger (*Zingiber officinale*) for three consecutive years. Yield was positively and significantly correlated with tillers/clump (r=0.83), internodal distance of rhizome (r=0.51) and plant height (r=0.50) and was negatively correlated with fibre content (r=-0.53). Karakai, Chekeralla, Rio-de-Janeiro, Thingpuri and Khonsa Local had high rhizome curing percentage (19.1-20.4%). The highest fibre content (7.6%) was recorded in Khasi Local and lowest in Nadia. However, Tura yielded highest (266.9 q/ha) followed by Poona (250.4 q/ha) and Basar Local (248.8 q/ha).

Singh *et al.* (1999) evaluated eighteen ginger cultivars for growth, yield and quality in Nagaland. Thinglaidum, Nadia and Khasi Local were tallest and had most tillers/plant. They also had the highest rhizome yields (more than 30 tonnes/ha). The lowest rhizome yield was recorded in Tura and HP 666 (less than 20 tonnes/ha). Thinglaidum, Nadia and Rio-de-Janeiro had the best fibre and oil contents.

Fifteen bold rhizome accessions were evaluated for yield and quality in multilocation trials. Based on the overall superior performance, accessions 35 and 107 were selected, multiplied and released for cultivation under the names IISR Rejatha and IISR Mahima, respectively (Sasikumar *et al.*, 2003).

2.1.2. Quality

Ginger

Natarajan *et al.* (1972) reported composition of ginger rhizomes taken from Kerala, as essential oil content (2.7%), acetone extract (3.9–9.3%), crude fiber (4.8–9.8%), and starch (40.4–59%).

According to Jogi *et al.* (1972), moisture content of ginger rhizome decreased with maturity. The maximum decrease of protein occurred 61/2 to 7 months after sowing. The maximum rise in the crude fibre and maximum decrease in the lipid content occurred in the ripening period between 51/2 and 6 months.

Krishnamurthy *et al.* (1972) evaluated the data on yield of volatile oil and oleoresin obtained from seven regions. Highest yield of volatile oil was obtained from the cultivar from Kalimpong and oleoresin from the cultivars from Nilgiri hills.

According to Jogi *et al.* (1978), fibre content ranged from 4.62 (cultivar Poona) to 6.98 per cent (cultivar Narasapattam). Cultivar Karakkal was lowest in dry recovery followed by cultivars Wynad local and Rio-de-Janeiro. Cultivar Rio-de-Janeiro had the highest oleoresin, whereas cultivar Karakkal had the highest oil. Crude fibre was least in cultivars Nadia and China.

According to Jayachandran *et al.* (1980), in Rio-de-Janeiro variety, yield of green ginger, oil and oleoresin content were highest at 7 months, where as dry ginger recovery and starch content were highest at 8 months maturity.

Nybe *et al.* (1982) found that cultivar Rio-de-Janeiro had the highest oleoresin content of (10.53%) followed by Maran (10.05%). Essential oil was highest in Karakkal (2.4%) and crude fibre was highest in Kuruppampadi (6.47%)

According to Sreekumar *et al.* (1982), dry ginger recovery ranged from 17.7 per cent in cultivar China to 28.00 per cent in cultivar Tura. Cultivars having more than 22% dry recovery (cultivars Moran, Jugijan, Ernad Manjeri, Nadia, Poona, Himachal Pradesh, Tura and Arippa) were found to be good for dry ginger production.

Akhila and Tewari (1984) reported percentage of volatile and non volatile extract for ginger from various countries. Volatile oil and non volatile extract for

Indian ginger was 2.2 and 4.25 per cent respectively. With respect to Sierra Leone and Jamaican ginger, volatile oil and non volatile extract were 1.6, 7.2 and 1.0, 4.4 respectively.

Haq *et al.* (1986) studied the composition of ginger from Bangladesh and found that rhizome contains essential oil (4%), ash (6.5%), proteins (12.3%), water-soluble proteins (2.3%), starch (45.25%), fat (4.5%) including free fatty acids, sterols (0.53%), cold alcoholic extract as oleoresin (7.3%), water solubles (10.5%), crude fiber (10.3%).and minerals (in g/100 g): Ca (0.025), Na (0.025), K(0.035), Fe (0.007), P(0.075), Mg (0.048), Cl(1.5ppm) and F(5.0ppm).

Baranowski (1986) studied the cv. Hawaii for 34 weeks and recorded the growth- related changes of the rhizome. The solid content of the rhizome increased throughout the season, but there was decline in the acetone extractable oleoresin content of dried ginger, the oleoresin content on fresh weight basis was roughly constant. The (6)-gingerol content of ginger generally increased with the age of the rhizome on a fresh weight basis. On a dry weight basis, gingerol generally exhibited a linear increase with maturity up to 24 weeks, followed by a steady decline through the rest of the period.

Mohanty and Panda (1991) studied induced mutation in ginger. Mutations were artificially induced in 5 ginger cultivars by employing one physical and three chemical mutagens. Twenty selected MV_3 generation mutants, along with the parental material, were compared in a 3-year yield evaluation. The highest yield was given by V_1K_1-3 (22.08 t/ha) followed by Suprabha (16.6 t/ha) and V_2E_5-2 (15.4 t/ha), in contrast to the parental cultivar UP (5.93 t/ha). The performance of 6 highest yielding lines, evaluated in a four year trial, confirmed the superiority of V_1K_1-3 (20.3 t/ha). Based on uniformly high yield, dry recovery, oleoresin and essential oil percentages, V_1K_1-3 mutant was recommended for release under the name Suravi during 1991.

According to Saika and Shadeque (1992), cultivars like Moran, Jorhat Hard, Thinladium and Wynad are having high fibre contents (7-8%). And not suitable as raw spices, cv.,Moran and Jorhat Hard are suitable for the extraction of oleoresins and volatile oils.

Goyal and Korla (1997) studied fresh yield, dry weight, essential oil, oleoresin and crude fibre contents of four ginger genotypes at different stages of rhizome development. In all genotypes, both fresh and dry weights of rhizomes increased steadily up to 225 days after planting. In fresh rhizomes, essential oil content peaked 210 days after planting, but essential oil as a percentage of dry weight decreased continuously throughout rhizome development. Oleoresin content on a dry weight basis declined until 210 days after planting, beyond which the contents increased in one genotype and remained unchanged in others. After an initial increase, crude fibre content on a dry weight basis decreased gradually until 210 days and then increased further with rhizome age. Except for the initial stage of rhizome development, the fresh rhizomes had the lowest level of crude fibre at 210 or 195 days in different genotypes. Harvesting between 225-240 days after planting is recommended to maximize the fresh yield, oleoresin content and recovery of dry ginger.

John and Ferreira (1997) evaluated five ginger selections under tropical conditions at Levubu area in South Africa and reported that there were significant differences ($P < 0.05$) in mass of fresh rhizomes, moisture and crude fibre content but not in the oleoresin and ginger oil content among the selections. G13 ranked first in respect of the mass of fresh rhizomes with high moisture content but lowest in the crude fibre content on wet basis. The selection G9 with a high crude fibre content of 6.8% on dry basis recorded the best results in terms of oleoresin (3.06%) and oil (0.52%) contents, however the dry ginger recovery was highest with G10 (27.5%). Thus, among the five selections studied G13 (Brazilian) gave better results for the early harvesting ginger industry (confectionery). For the drying and extraction

industries, respectively, the selections G10 (West Indies) and G9 (Taiwan) are preferable.

Makasone *et al.* (1999) claimed that gingerol contents of tetraploid strains were much higher than the diploids counterparts and they also showed that difference in pungency intensity between the diploids and the tetraploids, as evaluated by sensory test, were consistent with gingerol contents.

Korla *et al.* (1999) evaluated twenty-four ginger clones planted under rainfed and irrigated conditions at Solan, Himachal Pradesh for different quality attributes. Irrigation was applied 3 times at fortnightly intervals from the end of September to October in the irrigated plots. The analysis of variance indicated significant differences amongst the clones for DM, ginger oil, oleoresin and crude fibre contents, and yield per plot. However, growing conditions (rainfed and irrigated) exerted no significant effects except on crude fibre content and yield. DM content was highest in SG 692 (18.06 and 18.07% under rainfed and irrigated conditions, respectively) and least in SG 61 (12.90 and 13.05%). SG 61 registered high ginger oil (2.28 and 2.05%) and oleoresin contents (5.17 and 5.27%) but had high crude fibre content and poor yield. The highest yield (9.00 and 9.10 kg/plot) was obtained for Himagiri, the commercial cultivar, grown as a control; this was intermediate with respect to DM, ginger oil and oleoresin content but recorded the lowest crude fibre content.

Datta *et al.* (2003) studied quality of 12 ginger cultivars (Tanda, Rajgarh, Jughijan, Tura, Mazulay, Suprabha, Taffingiva, Suravi, Uttar Pradesh, Gorubathan, Bhoinse and local cultivar) grown in the subtropical humid region (Mondouri) of West Bengal, India. The dry recovery percentage was highest in Tura (26.77%) and lowest in Bhoinse (15.84%). Greater recovery (≥20%) was also recorded for Suravi (23.45%), Suprabha (20.60%), Uttar Pradesh (20.48%) and Gorubathan (20.30%). Suravi was superior in terms of oleoresin (10.3%) and essential oil (2.07%) contents.

Shankar (2003) evaluated seven induced variants along with three check varieties to exploit induced variability in ginger. Among the induced variants, autotetraploids Z-0-78 recorded the maximum driage (22.56%) whereas highest oil content was recorded in Z-0-86 (2.07%). Z-0-86 recorded the lowest fibre content (2.70%). With respect to oil yield per hectare, Rio-de-Janeiro registered the maximum value of 76.02 kg ha^{-1} followed by autotetraploid Z-0-86 (55.50 kg ha^{-1}). The colour of oil varied from light yellow to dark yellow. Sensory evaluation indicated that Rio-de-Janeiro had good sensory score ("++++") and the least preferred was Z-0-92 and Z-0-95 ("+"). With respect to oleoresin extracted with acetone and ethyl acetate, Z-0-86 recorded maximum content (9.16% and 7.74% respectively), whereas Rio-de-Janeiro gave the maximum oleoresin yield per hectare (280.15 kg ha^{-1} and 288.66 kg ha^{-1} respectively) followed by Z-0-86 (246.28 kg ha^{-1} and 207.97 kg ha^{-1} respectively). The colour of oleoresin extracted using acetone and ethyl acetate varied from pale brown to dark brown. Sensory evaluation of oleoresin indicated that Rio-de-Janeiro had most pleasing aroma with acetone and ethyl acetate as solvents. When extraction efficiency of solvents was compared, acetone was found to extract more oleoresin content (5.91%) than ethyl acetate (3.86%).

Ajay *et al.* (2013) studied the biochemical composition of two ginger varieties from Nigeria. Contents of crude fibre (21.90, 8.30), fat (17.11, 9.89), carbohydrate (39.70, 58.21), crude protein (12.05, 11.65), ash (4.95, 7.45) and moisture (3.95, 4.63) were reported in the two types respectively.

Yadav *et al.* (2014) studied effect of dates of planting on growth, yield and quality of ginger and reported that April 15th planting showed better oil content. Among spacings, the spacing of 35 cm × 25 cm gave highest dry recovery. The closer spacing of 15 cm × 25 cm recorded higher harvest index. It was observed that spacing had no significant effect on quality attributes viz., oil and crude fibre content. The treatment combination of 15th April planting and 35 cm × 25 cm spacing exhibited

higher dry recovery. The treatment combination of 15th April planting and 15 cm × 25 cm spacing showed maximum harvest index.

2.2 SOMACLONAL VARIATION

Somaclonal variation is the variation seen in plants that have been produced by plant tissue culture. Chromosomal rearrangements are an important source of this variation.

2.2.1 Yield

Samsudeen (1996) studied variability in ginger somaclones and found variability among somaclones in yield and yield attributes which resulted in identification of few promising high yielding lines with tolerance to rhizome rot.

According to Smith and Hamill (1996), adventious bud regenerants of ginger cultivar Queensland were more vigorous with more no of tillers /plant and lengthy pseudostem than conventionally propagated (CP) plants.

According to Pandey *et al.* (1997), conventially propagated plants of ginger cultivar Khin yai produced higher rhizome yield than adventitious bud regenerants. But rhizome of adventitious bud regenerants exhibited more branching indicating their yield potential.

Freitz *et al.* (2003) reported increase in tiller number, fresh and dry mass of shoots and roots in adventitious bud regenerants but rhizome yield and pseudostem length were more in control plants. Somaclones produced numerous small rhizomes with more number of fleshy roots and tuberous structures at the tips.

Sit and Malay (2007) reported that *in-vitro* regenerated plantlets performed better than the conventional planting materials and maximum yield/plant (356 g) was recorded from planting materials derived from *in-vitro* regenerated plantlets harvested from field.

Yang *et al.* (2009) also reported that tissue cultures plantlets had the advantages of rapid growth, strong growth vigor, disease resistance, strong adverse resistance. Its tubers are of bright yellow color, uniform size, heavy peppery, high quality, high yield with above 5000 kg/667 m^2.

Kurian (2010) tried induction of somaclonal variation in two polyploids (Z-0-78 and Z-0-86) and a diploid cultivar Himachal Pradesh. Evaluation of 289 somaclones (generated through indirect organogenesis and embryogenesis) indicated that somaclones were less tall with more number of tillers and higher mean yield when compared to control cultivars raised through bud culture. Ten per cent of somaclones produced rhizome yield more than 300g and the percentage yield increase over the control cultivars ranged from 92-148.

Resmi and Shylaja (2012) evaluated ginger somaclones for three consecutive years and revealed that somaclones were superior to conventionally propagated plants for various growth, yield and quality parameters. Twenty nine per cent somaclones were found superior to conventionally propagated plants in characters like height of pseudostem, number of tillers/plant, number of leaves/tiller and leaf area. For rhizome characters, 30% clones were found superior to conventionally propagated plants. Eighteen per cent somaclones exhibited superiority in yield over conventionally propagated plants giving a yield increase of 13%.

Black pepper

Sanchu (2000) studied variability in black pepper cultivar Cheriyakaniyakkadan derived through indirect organogenesis for morphological, yield and quality parameters. Variability was observed in leaf area, number of lateral branches, number of spikes per branch, spike length, number of berries per spike and recovery of essential oil and piperine.

Cardamom

Chandrappa *et al.* (1996) studied yield performance of tissue-cultured cardamom (*Elettaria cardamomum*) selections. Out of five selections, one selection had higher yields than control varieties i. e. Mudigere 1 and Mudigere 2, while the other lines had yields similar to the controls.

Sudharshan *et al.* (1996) compared plants regenerated by tissue culture from 8 high-yielding clones of cardamom (*Elettaria cardamomum*) with open pollinated progenies of these clones in field trials at 56 locations in Karnataka during 1988-1989. Clonal populations varied in the type of panicle, capsule shape and size and sterility. Overall variability in tissue-cultured plants was 4.5% compared to 3.0% in open pollinated seedling progeny.

Comparative study on yield performance of tissue derived plants and an open pollinated seedling of cardamom was carried out by Kuruvilla *et al.* (2005). Somaclones were superior to open pollinated seedlings in growth attributes such as number of tillers, bearing tillers, panicles per clump and yield. Irrespective of the seasons and location, 14 somaclones were identified with a yield potential of more than 750kg ha^{-1} under moderate management.

Large cardamom

Comparative study on growth and yield of adventitious bud regenerants and open pollinated seedlings of large cardamom was conducted by Rao *et al.* (2003). An increase of 1.5 times in yield contributing characters such as number of total tillers / clump, productive tillers/ clump, spike/clump and capsules/ spike and twenty times increment in yield were recorded in the somaclones as compared to open pollinated seedlings.

Vanilla

Madhusoodanan *et al.* (2005) assessed the performance of vanilla plants raised from tissue culture plantlets and vegetative cuttings on large-scale in planters' field covering a total area of 111 ha in Kerala, Karnataka and Tamilnadu. Observations on growth and yield attributes revealed that performance of tissue culture plants is on par with that of conventional plants raised from vegetative cuttings of comparable length. It is also reported that if good management practices are adopted, tissue culture plants perform better at the full bearing stage of the plant. This proves that tissue culture plants of vanilla can be popularized as a cost effective and faster source of planting materials compared to conventional vegetative cuttings.

Kacholam

Geetha *et al.* (1997) evaluated field performance of adventitious bud regenerants of *Kaempferia galanga* and *K. rotunda* for three seasons along with conventionally propagated plants. Somaclones were inferior in morphological characters and yield for first two seasons as compared to control plants but were on par with control plants in the third season.

2.2.2. Quality

Ginger

According to Ramachandran and Nair (1992), tetraploid lines from cultivars Maran and Mananthody gave lower essential oil content (2.3%) than the original diploid cultivar.

Mericlones of ginger cultivar Wynad local were comparable to the CP plants in the composition of starch, ash, acetone extract and volatile extract as reported by Bhagyalakshmi *et al.* (1994).

Zarate and Yeoman (1996) claimed that accumulation of [6] gingerol and [6] shogaol (phenolic pungent principles of ginger) was much higher in culture systems of *Zingiber officinale* where morphological differentiation was apparent. Cultures grown on a callus-inducing medium also accumulated these metabolites but to a lesser extent. There is a positive relationship between product accumulation and morphological differentiation, although unorganized callus tissue also seems to possess the necessary biochemical machinery to produce and accumulate some phenolic pungent principles. In contrast to earlier studies with the intact plant, there was no positive correlation between the amount of [6] gingerol accumulated and the number of pigmented cells in either of the culture systems investigated.

According to Rao *et al.* (2000), with respect to quality somaclones of Jamaican ginger were superior to the local ginger cultivar Kuruppapady in terms of oil and oleoresin recovery.

Smith *et al.* (2004) developed a tetraploid line named Buderim Gold, from Queensland a local cultivar. Tetraploid had compared the most favorably with 'Queensland' in terms of the aroma/ flavor profile and fibre content at early harvest, and had consistently good rhizome yield. The tetraploid had large rhizomes sections, resulting in a higher recovery of premium grade confectionery ginger and a more attractive fresh market product.

Paul (2006) reported somaclonal variation in two cultivars of ginger, Maran and Rio de Janeiro and found that the somaclones exhibited superiority over control plants in quality characters. Somaclones recorded higher dry recovery (19.73%) than conventionally propagated plants (16.02%). Of the two cultivars studied, higher driage was noticed in the clones of cultivar Maran (18.25%) than clones of cultivar Rio-de-Janeiro (15.62- 15.79%). In three clones of cultivar Maran viz. 488M, 110M and 970M, the driage recorded was very high registering driage values of 25, 22.56 and 22.50 per cent respectively. Recovery of essential oil varied between 1.00 to

2.50 per cent. Oil content was found high in somaclones of cultivar Rio-de-Janeiro (1.42 to 2.50%) than clones of cultivar Maran (1.00 to 2.25%). Oleoresin content ranged from 4.31 to 8.93 per cent in the somaclones evaluated. Higher recovery was noticed in clones of Rio-de-Janeiro (4.38 to 8.93%) than the clones of cultivar Maran (4.31 to 8.49%). Fibre content ranged from 1.96 to 6.86 per cent in the somaclones studied. Somaclones of cultivar Maran recorded low fibre content (1.96 to 5.24%) as compared to cultivar Rio-de-Janeiro (4.27 to 6.86%). Clones of cultivar Maran, M VI (1.96%) and 79 M (2.28%) showed the lowest crude fibre content.

Sanal *et al.* (2010) carried out study to explore variability between 18 diploids and tetraploids genotypes for their gingerol content. The teraploid ginger type was derived from the respective diploid ginger by shoot tip culture. (6)-Gingerol was the major pungent phenolic compound in all samples, while (8)-gingerol and (10)-gingerol occurred in lower concentration. The total gingerol content of the tetraploid type was much higher than that of the respective diploid type and especially (10)-gingerol type.

Shylaja *et al.* (2010) reported two new ginger varieties developed at Kerala Agricultural University, from cv. Maran, exploiting somaclonal variation. Athira is a high-quality cultivar suitable for fresh and dry rhizome, has low crude fibre contents and high zingiberene contents. Karthika is a high-yielding clone that produces highly pungent rhizomes rich in gingerol, suitable for the extraction of oleoresin

Reshmi and Shylaja (2012) reported that somaclones were superior over conventiotially propagated plant in attributes like dry ginger recovery, percentage of essential oil, oleoresin and fibre content.

Turmeric

Roopadarshini and Gayatri (2012) reported somaclonal variation in turmeric. Significantly high curcumin, oleoresin and volatile oil contents (%) were observed in

somaclonal variants when compared to the normal regenerants and also control plants.

Somaclonal variation in medicinal and aromatic plants

Geranium

According to Ravindra *et al.* (2004), somaclonal variants in *Pelargonium graveolens*, shows variability with respect to herb yield, essential oil content and oil components such as linalool and trans-rose oxide.

Patchouli

Ravindra *et al.* (2012) studied somaclonal variation in patchouli. Significant or highly significant somaclonal variation was observed for plant height, herb yield, essential oil content, essential oil yield, and contents of patchouli alcohol. The number of somaclones significantly superior to the parental variety for plant height, herb yield, essential oil content, and patchouli alcohol content in the essential oil and the maximum superiority over the parental variety for these traits ranged from 21-79%. Broad-sense heritability estimates of plant height, herb yield, and essential oil content were 0.60-0.70 while those of essential oil yield and patchouli alcohol content were 0.44 and 0.47, respectively.

Mint

Kukreja *et al.* (1991) reported wide range of somaclonal variation in Japanese mint (*Mentha arvensis*) for plant height (32.0-92.0 cm), leaf-stem weight ratio (0.53-2.32), herb yield (105.0-870.0g), oil content (0.32-1.10%) and oil yield (0.66-5.22 ml/plant). Variations were also recorded for 4 major constituents of the essential oil, i.e. menthol (65.2-94.77%), menthone (1.40-20.89%), isomenthone (0.96-5.14%) and menthyl acetate (0.75-8.52%).

Kukreja *et al* (1992) reported wide range somaclonal variation in *Mentha arvensis var. piperascens* for menthol (65.2-94.8%), menthone (1.4-20.9%), isomenthone (1.0-5.2%) and menthylacetate (0.8-8.5%).

Xue *et al.* (1998) reported somaclonal variation in peppermint (*Mentha arvensis* L.) for some agronomic traits such as fresh weight per plant, flowering date, stem branches, number of falling leaves and mint oil content. Some somaclones exceeded controls in oil and menthol contents by 27.77% and 8.16-10.86%, respectively.

Kukreja and Dhawan (2000) evaluated 27 mint somaclones in a replicated plant to row trial with parent plant CIMAP/Hy-77 as the control. Two somaclones Sc 59 and Sc 179 selected on the basis of higher herb yield recorded 55.8% and 64.3% increase and somaclones Sc 93, Sc 114, Sc 121, Sc 124 that were selected on the basis of better oil content exhibited 47.2%, 50.6%, 57.5%, and 48.2% increase respectively, in oil yield over the parent control. A decline in menthol content along with 2-4 fold increase in menthone and isomenthone was observed. Somaclones Sc 93 and Sc 179 showed stability in terms of per cent composition of the volatile oil.

Aloe

Saggoo and Ramandeep (2010) compared the morphological and biochemical characters of tissue culture derived and field grown clones of one year from two different accessions of *Aloe vera*. Comparison of somaclones with the parental clones showed variation in size of plants, size of leaves, spines, etc. The callus regenerated plants of HPM1 were bigger in size than the parental clones and showed marginal increase in the amount of carbohydrate, protein, chlorophyll and phenol contents over the control plants. There was decrease in aloin content and juice quantity but increase in gel content in the somaclones. The tissue culture raised plants of PBL3 were smaller in size and exhibited decreased amount of carbohydrate, protein, chlorophyll,

aloin, juice and gel contents than the parental clones but have increased amount of phenols.

Citronella

Mathur *et al.* (1998) evaluated 19 somaclones from variety Jorhat for 7 agronomic traits, viz. herbage yield, tiller number, diameter of the length and area of the longest leaf, fresh and dry weight ratio and oil content. In addition, variations were also recorded for 6 major constituents of the essential oil, i.e. citronellal, citronellol, geraniol, citronellyl acetate, geranyl acetate and elemol. Correlation analysis between agronomic traits revealed a significant negative correlation between oil content and herbage yield.

Ashwagandha

Satiander *et al.* (2012) studied variation among 54 regenerated plants attained through indirect organogenesis from leaf explants. Organogenic calli were induced on Murashige and Skoog medium containing 2 mg l^{-1} kinetin and 1 mg l^{-1} indole-3-butyric acid. High-performance liquid chromatography was used for quantitative determination of the major withanolides in the somaclones. One somaclone (WS-R-1) showed significantly higher accumulation of 12-deoxywithastramonolide (WS-12D; 0.516%) compared to the explant donor mother plant (0.002%).

2.3 CHEMOPROFILING OF VOLATILE OIL AND PUNGENT PRINCIPLES

2.3.1 Chemoprofiling of volatile oil

Three ginger oils obtained by different extraction methods were analysed qualitatively and quantitatively by GC/MS. In the three oils, 46, 50 and 61 compounds were identified. The main components and contents of each oil were clearly different. The main components of the steam-distilled oil were monoterpenes and sesquiterpenes; the pungent components were not found. Besides sesquiterpenes,

the other 2 oils contained mainly the pungent components; the total content was 18.61% in the cold-pressed ginger oil and 23.09% in the supercritical CO_2-extracted ginger oil. These 2 oils preserved the typical spicy odour and pungency of ginger (Yu *et al.*, 1998).

Onyenekwe and Hashimoto (1999) studied composition of the essential oil by GC and GC-MS techniques. The oil yield was 2.4% and the oil consisted of 64.4% sesquiterpene hydrocarbons, 6.6% carbonyl compounds, 5.6% alcohols, 2.4% monoterpene hydrocarbons and 1.6% esters. The main compounds were zingiberene (29.5%) and sesquiphellandrene (18.4%). A number of constituents not previously reported in ginger oil were identified. These included 2,6-dimethyl hepten-l-ol, α-gurjunene, linalool oxide, isovaleraldehyde, 2-pentanone, cadinol, α- and γ-calacorene, eremophyllene, t-muurolol, α-himachallene, α-cubebene acetic acid, pinanol, α-santalene, geranyl propionate, geranoic acid, (E,E)-α-farnesene, n-methyl pyrrole and geranic acid.

Essential oil extracted from ginger rhizomes using different solvents was analysed by GC-MS. Principal constituents detected in the three extracts were terpenes, but the composition and contents of the terpenes were different. The contents of total terpenes and sesquiterpenes increased with increasing polarity of the solvents used for extraction, but the content of monoterpenes decreased. The contents of unsaturated sesquiterpenes with antioxidant activity increased with increasing polarity of extracting solvents. It is shown that the antioxidant activity of ginger extracts increases with increasing polarity of solvents used for extraction (He *et al.*, 2001)

Martins *et al.* (2001) studied essential oil composition of three Zingiberaceae widely used as medicinal and aromatic plant from S. Tomé and Príncipe: *Aframomum danielli, Curcuma longa* and *Zingiber officinale*. Two samples of the essential oils from fruit of *Aframomum danielli* and from rhizomes of the other two species, were

obtained by hydrodistillation and analysed by GC, GC-MS, and C-NMR. The essential oil from fruits of *Aframomum danielli* has been studied for the first time and was characterized by its high content of monoterpenes, with 1,8-cineole [eucalyptol] (25.5-34.4%) the major constituent, followed by β-pinene (14.1-15.2%) and α-terpineol (9.9-12.1%). Essential oils from the rhizomes of *Curcuma longa* contained a lower content of *ar*-turmerone (4.0-12.8%) than those reported in the literature for *Curcuma longa* from other origins (24.7-31.4%), whereas the results for *Z. officinale* essential oils were in accordance with the literature data.

Sharma *et al.* (2002) analysed essential oil of ginger by high resolution gas chromatography and GC-MS and report that 47 compounds of which the major ones were ar-curcumene (15.6%), geraniol (14.5%), neral (11.6%) and 1,8-cineole (11.8%).

Wossa *et al.* (2004) analyzed essential oil composition of ginger along with other spices by a combined gas chromatography-mass spectrometry (GC-MS) method. The results indicated that citral (18.4%), zingiberene (16.8%) and camphene (11.2%) were the main constituents of ginger oil.

Pino *et al.* (2004) studied essential oil composition of rhizomes from Cuba by using GC-MS technique and reported that, ar- curcumene (22.1%) was major component, followed by cadina-1, 4-diene (12.5%), zingiberene (11.7%), β-bisabolene (11.2%).

The hydrodistilled essential oil of ginger rhizomes from three different geographical locations in India, Mizoram, Chennai (Tamil Nadu) and Sikkim, were analysed by GC and GC-MS. Zingiberene (10.5-16.6%) was the major constituent in all essential oils. Quantitative differences in the concentration of the constituents were observed. The GC and GC-MS analysis of Mizoram, Chennai and Sikkim (Majhauley and Bhainsey varieties) ginger rhizome oils resulted in the identification

and quantitation of 29, 29, 28 and 28 constituents representing 84.3, 86.6, 88.8 and 86.8% of the total oils, respectively. Among the 4 oils analysed, the Majhauley variety showed a higher content of zingiberene (16.6%) followed by e-citral (12.0%), z-citral (8.8%), camphene (7.6%) and ocimene (6.5%) (Raina *et al.*, 2005).

Wohlmuth *et al.* (2006) analysed essential oil composition of 17 clones of Australian ginger, including commercial varieties and experimental tetraploids clones by gas chromatography-mass spectrometry (GC-MS). The essential oils of 16 of the 17 clones were found to be of similar composition. These oils were characterized by very high citral levels (51-57%) and relatively low levels of sesquiterpene hydrocarbons. The citral levels of most of these oils exceded those previously reported for ginger oils. The neral to geranial ratio was shown to be remarkably constant across all 17 somaclones. Among the rhizomes, one clone, the cultivar Jamaican yielded oil with substantial different composition, lower citral content, higher levels of sesquiterpene hydrocarbons, and also significant higher concentration of pungent principles.

Menon *et al.* (2007) studied the essential oil composition of fresh and dried ginger by GC/MS. It was found that geranial (24.2%) and zingerone (14.2%) were the major compounds in the original aroma of fresh ginger and their contents decreased during processing. It was observed that the hydrocarbon content of the oil increased and the oxygenated compounds decreased as ginger was processed into dry ginger and ginger oil.

Ginger essential oil from hot air drying was extracted by simultaneous distillation and solvent extraction (SDE). Qualitative and quantitative analysis were done by GC-MS. The highest content was zingiberene (18.99%), followed by β-sesquiphellandrene (14.35%), α-farnesene (9.95%), α-curcumene (8.77%), and β-bisabolene (5.63%). These 5 components accounted for 57.69% of the total

components and were identified as the main chemical component of ginger essential oil (Luo and Lu, 2010).

Indu *et al.* (2012) studied the volatile oil composition of two most popular cultivars of Sikkim namely, Bhaisa and Majulay by GC-MS. Sixty constituents accounting for 94.9% and 92.6% of the Bhaisa and Majulay oils were identified. The major compounds of Bhaisa oil are geranyl acetate (18.8%), zingiberene (16.3%) and geranial (8.2%) and those of Majulay oil were zingiberene (19.8%) and geranial (16.5%). Compared to other ginger cultivar oils, the Bhaisa oil had higher content of oxygenated compounds (43.1%).

Kiran *et al.* (2013) studied essential oil composition of seventeen cultivars from North-East India by gas chromatograph (GC) and GC-mass spectrometry (GC-MS). Major volatile constituents were camphene (8.49±0.41%), neral (4.95±0.34%), geranial (12.36±0.46%), zingiberene (20.98±2.34%) and β-sesquiphellandrene (7.96±0.66%). Assam fibreless cultivar showed highest yield of essential oil (4.17±0.05%) and higher monoterpene hydrocarbon content (38.65±0.11%) than sesquiterpene hydrocarbon (25.38±2.3%), which is unique among all cultivars. Assam Tinsukia had the highest citral content (23.66±1.60%) and Meghalaya mahima had the highest zingiberene content (29.89±0.42%).

Zhang *et al.* (2014) studied the components of ginger oil with two different extraction methods. Ginger oil was extracted by ultrasonic assisted multi-enzyme hydrolysis method and ultrasonic-assisted extraction method from ginger powder. The components of ginger oil were analyzed by gas chromatography-mass spectrometry (GC-MS). The average yields of ginger oil with two methods were (6.72±0.02 %) and (6.60±0.04 %) respectively. Fifty three components were identified by ultrasonic assisted multi-enzyme hydrolysis method, and the content of gingerol was 25.36%, zigiberene was 18.12%. Forty four components were identified by ultrasonic-assisted extraction method, and the content of zigiberene was 24.41%,

gingerol was 20.14%. There was difference in the components of ginger oil betweer the two methods.

2.3.2 Chemoprofiling of pungent principles

Chen *et al.* (1986) analysed pungent gingerol compounds in green (4–5 months) and dry (8–9 months) ginger by high performance liquid chromatography (HPLC) on a reverse phase column (RP-18) and reported total gingerol content (6-, 8- and 10-gingerol) of green ginger was ranged from 0.65–0.88% (w/w) while dry ginger varied from 1.10–1.56% (w/w).

Gurdip *et al.* (2005) analyzed the fresh rhizome essential oil and oleoresin of ginger by GC-MS and reported the presence of 69 components, accounting for 96.93% of the oil. The major component was α-zingiberene (28.62%) followed by camphene (9.32%), *ar*-curcumene (9.09%) and β-phellandrene (7.97%). Analysis of the oleoresin showed the presence of 34 components, accounting for 88.63% of the oleoresin. The major components were *trans*-6-shogaol (26.23%), *trans*-10-shogaol (13.0%), α-zingiberene (9.66%) and 10-gingerdione (6.80%).

The essential oil and oleoresins (ethanol, methanol, CCl_4 and isooctane) of ginger were subjected to GC-MS analysis. Geranial (25.9%) was the major component in essential oil; eugenol (49.8%) in ethanol oleoresin, while in the other three oleoresins, zingerone was the major component (33.6%, 33.3% and 30.5% for methanol, CCl_4 and isooctane oleoresins, respectively). The antioxidant activity of essential oil and oleoresins were evaluated against mustard oil by peroxide, anisidine, thiobarbituric acid (TBA), ferric thiocyanate (FTC) and 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods. They were found to be better antioxidants than butylated hydroxyanisole (BHA). The antimicrobial properties were also studied using various food-borne pathogenic fungal and bacterial species. The essential oil and CCl_4 oleoresin showed 100% zone inhibition against *Fusarium moniliforme*. For other tested fungi and bacteriae, the essential oil and all oleoresins

showed good to moderate inhibitory effects. Though, both essential oil and oleoresins were found to be effective, essential oil was found to be better than the oleoresins (Gurdip *et al.* 2008).

Zhan *et al.* (2008) studied the ginger oleoresin composition by gas chromatography and mass spectrometry technique. The study identified both volatile and non-volatile compounds. The volatile compounds were mainly α-zingiberene (22.29%), β-sesquiphellandrene (8.58%), α-farnesene (3.93%), β-bisabolene (3.87%), α-curcumene (2.63%), which mostly consisted of sesquiterpene hydrocarbons. The pungent compounds of ginger were mainly 6-gingerol (9.38%), 6-shogaol (7.59%), zingerone (9.24%) produced by the thermal degradation of gingerols or shogaols.

More than 100 compounds have been reported from ginger, some of which are isolated and characterized. Others are tentatively identified by GC-MS and/or LC-MS. [6]-Gingerol, the major gingerol in ginger rhizomes, has been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic, and cardiotonic effects (Kubra and Rao, 2012).

Li Xia *et al.* (2012) employed gas chromatography-mass spectrometry technique to analyse the ginger oleoresin products extracted from ginger by supercritical CO_2. More than 30 components were detected under the optimization conditions. α-Curcumene (3.69%), zingiberene (17.11%), α-farnesene (2.28%), β-bisabolene (5.12%) and β-sesquiphellandrene (7.77%) were the main active sesquiterpene hydrocarbon components. The pungent compounds were mainly gingerol (7.70%) and zingerone (30.36%) produced by the thermal degradation of gingerols in the analysis.

2.4 PREPARATION OF VALUE ADDED PRODUCTS

2.4.1Ginger candy

Anis *et al.* (2012) conducted experiment to develop preserve and candy from fresh ginger and studied their storage life. The preserve was made from 60%, 65% and 70% sugar concentration. The candies were made from 65%, 70% and 75% sugar concentration. Among them, the best preserve and candy were identified on the basis of overall acceptability. The study showed that the color, flavor, texture and overall acceptability among the preserves and among the candies were different. The preserve (GP70) made from 70% and the candy (GC75) made from 75% sugar concentration was best among others of the similar product. Higher concentration of sugar and slower processing gives higher acceptability for preserve and candy. Among different changes, moisture concentration was prominent during preparation of preserve and candy. The moisture content was 42.0% and 37% for preserve and candy respectively which were nearly half of the initial concentration of fresh ginger. The storage stability of candy (90 days) was higher than storage stability of preserve (60 days).The color, flavor and fungal growth of candy were acceptable as there were no changes up to 90 days of storage. The remarkable change was noticed at 120 days of preservation and the candy remarked as unacceptable to consume. The changes occurred possibly due to fermentation in presence of fungus (Anis *et al.*, 2012).

Nath *et al.* (2013) reported standard protocol for ginger candy preparation. The experimental parameters considered were slice thickness (5.0-25.0 mm) and blanching duration (10-30 min) followed by dipping in 40°B and 75°B sugar solutions containing 2.0% citric acid respectively, for 1 and 2 h at 95°C and dried at 60°C for 1 h. RSM design was considered for this experiment and final products were evaluated for their textural properties, TSS, acidity, TSS:acid ratio, taste score and overall acceptability. The optimum product qualities in terms of hardness (2.08 kg), TSS (73.4%), acidity (1.31%); TSS:acid ratio (56.3), taste score (7.98) and overall acceptability (8.07) were obtained for slice thickness of 10.9 mm and blanching time of 24.9 min.

Sivakumar (2013) made attempt to standardize amla sweet candy with different blanching time viz., 5, 10 and 15 minutes. The prepared sweet candies were standardized on the basis of sensory evaluation. Among these, a candy prepared with 10 minutes blanching time treatment was found to be the best.

Babariya *et al.* (2014) standardized a recipe for the preparation of candy (tuti fruiti) from unripe papaya. The experiment consisted of 18 treatments *viz.*, NaCl (10%, 20%, 30% and 40%), $Ca(OH)_2$ (0.5% and 1.0%) and sea water (15 ppt, 25 ppt and 35 ppt) and each level with sugar (add direct sugar and add 50 °Brix sugar syrup). The results indicated that the quality observations and sensory evaluation affected by various treatments. The treatment T_3 (20% NaCl and add direct sugar) recorded the maximum total sugar, non reducing sugar and sensory evaluation (colour, texture, flavour, taste and overall acceptability) during entire period of storage (Six months).

2.4.2 Ginger paste

Baranowski (1985) and Giridhar *et al.* (1996) recommended a process temperature of 80^0C for ginger–garlic paste with a pH of approximately 4.0. The combination of antioxidant stabilizer and preservative was very important for the preparation of a high-quality ginger–garlic paste.

Fresh garlic bulbs of 16 weeks maturity were procured locally and stored at 25^0 C for one month before processing. The garlic bulbs were subjected to mild pressure by hand to separate into cloves. Cloves were dried in a tray drier at 40^0C for 30 min to facilitate peeling. Peeling was done manually. After peeling, cloves were blanched at 90^0C for 15 min in water followed by grinding in a laboratory size grinder. The ground material was passed through a 14-mesh sieve to obtain a product of uniform consistency. The yield of the ground material was 90%. Desired quantity of sodium chloride (w/w) was added to increase the total solids (TS). The final pH

was adjusted to 4.1 by adding 30% citric acid (w/v) solution. The paste was pasteurized at selected temperatures (70, 80 or 90^0C) in a stainless steel container by heating in a constant temperature water bath and was continuously stirred to achieve uniform heating. Pasteurization was carried out for 15 min once the temperature at the geometric center reached to the desired level and immediately filled in presterilized glass bottles. The packed bottles were cooled in forced air and stored at 25^0C (Jasim *et al.*, 2000).

Jasim and Shivhare (2002) reported preparation of paste by mixing onion, ginger and garlic with 10% common salt and citric acid. The paste was thermally processed at 80°C for 15 min and packed in glass bottles and polyethyleneterephthalate (PET) containers. Mixed paste samples were stored at 25±1°C.

The ginger paste was prepared by adding common salt at 8% (w/w) to ginger puree to increase its total soluble solids (TSS). Fresh ginger puree had a pH of 6.38 and was adjusted to 4.05 by adding 30% citric acid solution (w/v). It has been established that an acidified food (pH < 4.6) requires only pasteurization to retain its fresh spice odour. The paste was therefore, thermally processed at 80^0C for 15 min and packaged immediately (Jasim, 2004).

Shaista *et al.* (2009) evaluated the effect of five stabilizers i.e. citric acid, sodium metabisulfite, sodium benzoate, olive oil and ascorbic acid mixed in the ginger-garlic paste against five pathogens (*E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis* and *Enterobacter aerogenes*). Activity of the control paste decreased during storage. Antimicrobial activity of the paste was stabilized by various stabilizers when incorporated. Sodium metabisulfite, olive oil and ascorbic acid were found to be effective to stabilize the antibacterial activity of the paste considerably. *E. coli* and *Salmonella typhi* showed more resistance in case of citric acid and sodium benzoate provided in the paste.

Ginger and garlic paste was prepared by mixing 1:1 (w/w) proportion of ginger and garlic along with sodium chloride (1%). The pH of the paste was adjusted to 4.5 by the addition of citric acid. Xanthan gum (2 g/L) and sodium benzoate (0.2 g/L) were added to the paste and then filled in retort pouches. The filled pouches were subjected to thermal processing at 85^0C with a holding time of 2 at 80^0C at the centre of the paste (Priya *et al.*, 2010).

Lakshmi *et al.* (2015) reported about preparation of ginger paste. Ginger paste was prepared by washing, peeling and pulverizing of ginger rhizomes to form smooth paste and was mixed with ascorbic(100 mg kg^{-1}) and citric (100 mg kg^{-1}) acids and subsequently packed in polyethylene pouche (25µm thickness, 50g pack size).

2.5 QUALITY PARAMETERS

2.5.1 Physico –chemical parameters

2.5.1.1 *Moisture*

Tirupathi *et al.* (1998) evaluated aonla dried to a moisture content of 7.20 per cent by using a solar dehydrator and reported a progressive decline in moisture during storage of 135 days.

According Sivakumar (2013) there was decrease in moisture content during storage in amla sweet candy. Sagar *et al.* (2000) observed a rapid change in moisture content of mango powder up to two months of storage at ambient and low temperatures.

2.5.1.2 *Colour*

Zainun (2001) reported that ginger paste was found to have a fair shelf life under refrigeration but degradation of colour, odour and taste made the ginger paste unacceptable one week after storage at ambient temperature.

Priya *et al.* (2010) reported slight decrease in green color with the addition of salt whereas the addition of citric acid alone substantially increased the greening of ginger–garlic paste.

Priya *et al.* (2010) reported that in ginger paste color values a, and b (green and yellow) decreased, whereas the *L* (lightness) values increased with decrease in pH. Paste behaved like a non-Newtonian fluid, and exhibited shear-thinning behavior. Physicochemical and microbial properties did not show significant changes during storage.

2.5.2 Biochemical parameters

2.5.2.1 *TSS*

Sivakumar (2013) reported that in amla sweet candy total soluble solids (TSS) and total sugar decreased from 58.20 to 57.10 °B and 45.74 to 45.13 per cent respectively, after nine months of storage.

Mishra *et al.* (2013) found that in bael candy the percentage total soluble solids (TSS) increased gradually during storage in both types of packaging containers. However, the rate of change of TSS was faster in glass jars than polythene pouches. At the initial stage, the TSS value was 73.8% in both types of containers but at the end of observation (8 month after storage), the TSS of candy stored in glass jar was 78.5 % while in polythene pouch it was 75.2 %.

2.5.2.2 *PH*

Jasim *et al.* (2000) reported that garlic paste contained 33% total solids, 9.6% sodium chloride and 0.35% titratable acidity while pH and water activity values were 4.1 and 0.86, respectively. The Hunter color L^*, a^* and b^* values of the paste were 58.26,-9.54 and 20.96, respectively.

Jasim (2004) reported that pH and acidity of ginger paste were 4.05 and 0.32% (in terms of anhydrous citric acid), respectively. Total soluble solids (TSS) and total solids were 11.6 Brix and 15.72%, respectively. The paste contained 7.76% sodium chloride. The Hunter colour L, a and b values of prepared ginger paste were 59.93, 2.01 and 22.95, respectively.

Both high pressure and thermally processed paste samples demonstrated a non-significant decrease in pH throughout refrigerated storage. (Lakshmi *et al.*, 2015).

2.5.2.3 ***Titratable acidity***

Rejano *et al.* (1997) and Ahmed *et al.* (2001) have reported that TSS, sodium chloride, titratable acidity and pH of ginger paste did not change significantly ($P > 0.05$) during storage.

A decreasing trend in the acidity of dehydrated aonla during storage as observed by Tirupathi *et al.* (1988)

Mishra *et al.* (2013) observed that the per cent TSS, acidity and browning of bael candy increased while ascorbic acid decreased during storage in both types of containers. The organoleptic quality of candy was extremely good in polythene pouches up to 4 months while only one month in glass jar.

2.5.2.4 ***Non enzymatic browning***

Sawamura *et al.* (1991) reported that browning in citrus fruit juice increased with increase in storage temperature and it was intensely brown at 37°C but no browning was seen in juice stored at 2°C.

According to Pandey and Singh (1999), in guava RTS beverage, the browning (O. D value at 440 nm) increased from 0.00 to 0.09 during 6 months of storage at ambient temperature.

A gradual increase in browning of bael candy was noticed in both types of containers and the O.D. value increased from 0.02 to 0.07, though the differences between O.D. values of both types of containers were marginal. The browning of candy was mainly due to non-enzymatic reactions such as organic acid with sugar and /or oxidation of phenols, which lead to formation of brown pigments (Mishra *et al,* 2013).

2.5.2.6 *Poly phenol oxidase activity*

PPO is thermo-tolerant and active in a broad pH range (pH 3.5 to 8). Heat inactivation studies showed a decrease in enzyme activity at 75°C and above. Lower concentrations of MgCl2 (1 mM) and CaCl2 (0.5 mM) activated the enzyme whereas higher concentrations (10 mM) reduced the activity. L- Cysteine HCl, L-ascorbic acid, potassium metabisulfite and Na Cl inhibited PPO strongly (Ancy *et al.,* 2011).

2.5.3 Microbial quality

Jasim *et al.* (2000) reported that total plate count (TPC) and lactobacillus count of the ginger paste before thermal processing were 16.7 x 10^{-3} and 4160, respectively; while the coliform and yeast and mold counts were less than 10 and 100, respectively. Thermal processing of paste at 90^0C for 15 min reduced TPC to 100 while lactobacillus, coliform, yeast and mold were found to be negative. The TPC value increased from 100 to 500 CFU/g where as colifom was negative and lactobacillus and yeast and mould counts increased from nil to less than 100 during 6 months storage at 25^0C.

Menon (2000) reported low microbial counts in dehydrated fruits and vegetables, dried to moisture content less than 3 per cent after blanching and drying.

High pressure treatment and thermal treatment were equally effective in reducing microbial counts in the giner paste. The addition of citric acid is also found

to inhibit microbial growth by causing intra cellular acidification (Nielsen and Arneborg, 2007).

Priya *et al.* (2010) reported that in fresh ginger–garlic paste initially total plate count (TPC) was 2 x 10^{-2} colony-forming unit (cfu)/g, whereas the coliform and yeast and mold counts were below 10 and 100 cfu/g, respectively. Thermal processing of paste at 85^0C for 2 and 5 min. reduced TPC to 65 while coliforms, yeast and mold were found to be nil. Addition of sodium benzoate (200 ppm) helped in controlling microbial load completely.

According to Sivakumar (2013), amla sweet candy showed a very slight increase in microbial load during the storage period. The initial bacterial, fungal and yeast counts were 1x10^{-3}g, 1x10-^{2}g and 1x10-^{2}g respectively, which had increased to 4x10-^{3}g bacterial counts, 3x10-^{2}g fungal count and 3x10-^{2}g yeast count at the end of the storage period.

2.5.4 Sensory evaluation

Sivakumar (2013) reported that in amla sweet candy, organoleptic scores slightly decreased with advancement in the storage period. The organoleptic scores of colour, appearance, texture, taste and overall acceptability were 8.9, 8.8, 8.6, 8.8 and 8.7 respectively at initial study period. After nine months of storage the organoleptic scores of amla sweet candy was recorded as 8.0, 7.9, 7.8, 8.0 and 7.9 for colour, appearance, texture, taste and overall acceptability, respectively.

Mishra *et al.* (2013) reveal that the acceptability of bael candy stored in polythene pouch was up to 4 months and in glass jar it was only one month without any change. The acceptable and marketable quality of candy was up to 4 months in glass jar while up to 8 months in polythene pouch. Overall, better organoleptic quality of candy was also rated high in polythene pouch than the candy stored in glass jar.

Lakshmi *et al.* (2015) reported that ginger paste treated at 600MPa had the highest flavour and taste, with overall acceptability score of 7.8; the next being 400MPa with acceptability score of 7.3 followed by 200MPa treated paste with a score of 6.5.

MATERIALS AND METHODS

MATERIALS AND METHODS

The investigations on "Screening somaclones of ginger (*Zingiber officinale* Rosc.) for value addition" were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Thrissur, during the period May 2013 and June 2015. Main aspects focused in the study are as follows:

1. Evaluation of somaclones of ginger for variations in quality attributes and identification of novel chemotypes through chemoprofiling
2. Screening somaclones of ginger for product diversification and identification of elite types for value added products

3.1 EVALUATION OF SOMACLONES

3.1.1 Experimental material

Forty Somaclones developed through indirect methods of regeneration from two induced polyploids of ginger (Z-0-78 from Himachal Pradesh treated with 0.25% colchicine by injection method and Z-0-86 from Rio-de-Janeiro treated with 0.1% colchicine by hole method) and diploid cultivar Himachal Pradesh formed the experimental material (Table 1).

These somaclones were maintained as part of DBT funded project entitled "Exploitation of somaclonal variation for disease tolerance and high yield in ginger" at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara. The rhizomes were harvested at full maturity when the leaves withered, rhizome yield recorded and quality attributes studied.

Table 1. Details of somaclones selected for the study

S. No.	Parents	Somaclones	Mode of regeneration
1	Z-0-78	C 78 13	Indirect organogenesis
2		C 78 116**	
3		C 78 284	
4		C 78 381	
5	Z-0-86	C 86 8	
6		C 86 23	
7		C 86 26	
8		C 86 32	
9		C 86 40	
10		C 86 82	
11		C 86 124	
12		C 86 139	
13		C 86 141	
14		C 86 191	
15		C 86 201**	
16		C 86 261**	
17	Himachal Pradesh	CHP 87**	
18		CHP 99	
19		CHP 118	
20		CHP282	
21	Z-0-78	SE 78 12	Indirect embryogenesis
22		SE 78 26	
23		SE 78 30	
24		SE 78 174	
25	Z-0-86	SE 86 24	
26		SE 86 26	
27		SE 86 40	
28		SE 86 41**	
29		SE 86 42	
30		SE 86 81	
31		SE 86 83	
32		SE 86 131	
33		SE 86 142	
34	Himachal Pradesh	SE HP 8	
35		SE HP 9	
36		SE HP 64	
37		SE HP 73	
38		SE HP74	
39		SE HP129	
40		SE HP 146	

** Mutants (10 Gy γ rays)

3.1.3 Rhizome yield

3.1.3.1 *Fresh rhizome yield*

The harvesting of rhizomes was done eight months after planting by uprooting individual clumps. Yield per plot (kg) was recorded and yield per hectare was computed (tonnes).

3.1.3.2 *Dry rhizome yield*

The per hectare yield of dry rhizomes (tones) was computed from the per hectare fresh yield and driage per cent.

3.1.4 Quality attributes

Rhizomes of the selected 40 somaclones, replicated twice, were analyzed for quality attributes such as driage and contents of volatile oil, oleoresin, starch and crude fiber.

3.1.4.1 *Dry recovery*

One kilogram of fresh rhizome was rough peeled and sun dried initially and later in hot air oven (55^0C) till a constant weight was obtained. Dry recovery of rhizome was expressed in percentage.

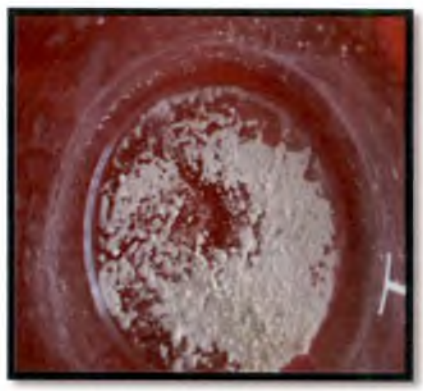
3.1.4.2. *Estimation of volatile oil*

Volatile oil was estimated by water cum steam distillation method using Clevenger apparatus as per AOAC (1980). The recovery of volatile oil was expressed as percentage. Twenty five grams of coarsely ground powder from each somaclone was distilled for three hours for estimation of volatile oil. From the dry rhizome yield per hectare and oil content, oil yield per hectare was computed.

Dried rhizome

Volatile oil

Oleoresin



Starch

Crude fibre

Plate 2. Quality attributes of ginger somaclones

3.1.4.3 *Estimation of oleoresin*

The content of oleoresin in the samples were estimated using Soxhlet method of extraction as per AOAC (1980). Five grams of powdered sample was refluxed with 125 ml of acetone. Extraction was continued till the solvent became colourless. The acetone extract of the sample was transferred to a pre- weighed beaker and the solvent was evaporated and weight of the beaker along with the extract was recorded. The recovery of oleoresin was expressed in percentage. From the dry rhizome yield per hectare and oleoresin content, oleoresin yield per hectare was computed.

3.1.4.4 *Estimation of crude fibre*

The content of crude fiber was estimated as per Sadasivam and Manickam (2010) and expressed in percentage. Two gram of ginger powder was boiled with 200 ml of sulphuric acid for 30 minutes, filtered through muslin cloth and washed with distilled water to remove acidic nature. Subsequently boiled with 200 ml of sodium hydroxide solution for 30 minutes and it was again filtered and washed with 25 ml alcohol. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The content of crude fibre was given by the difference in weight and expressed as percentage (Plate 3).

3.1.4.5 *Estimation of starch*

Starch was estimated colorimetrically using anthrone reagent, as suggested by Sadasivam and Manikam (2010). Weighed 0.3 g of the sample and extracted with 80 per cent ethanol to remove sugars. Residue was repeatedly extracted with hot 80 per cent ethanol to remove the sugars completely. The residue was dried over a water bath and added 5 ml water and 6.5 ml of 52 per cent perchloric acid and extracted in the cold for 20 minutes. Centrifuged the sample and re-extracted with fresh perchloric acid. The supernatant was pooled and made up to 100 ml. Pipetted out 0.2 ml of the

Ginger powder + Sulphuric acid

Residue

After ignition

Plate 3. Steps in crude fibre estimation

supernatant and made up to one ml with water and added 4 ml of anthrone reagent, heated for 8 minutes, cooled and read the OD at 630 nm.

A standard graph was prepared using serial dilutions of standard glucose solution. From the graph, glucose content of the sample was obtained. The glucose content was multiplied by a factor of 0.9 to arrive at the starch content (Plate 4).

3.1.5 Chemoprofiling of volatile oil and non volatile pungent principles

3.1.5.1 *GC-MS analysis of ginger volatile oil*

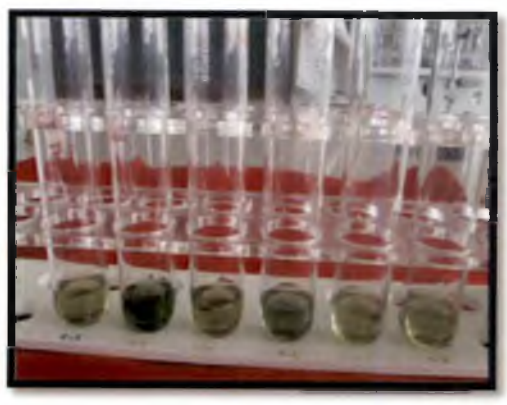
The volatile oil from dried rhizomes of 11 ginger somaclones were analyzed using a Shimadzu GC-2010 gas chromatograph equipped with QP 2010 mass spectrometer and RTX–5 column (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.67 ml min^{-1}. The injection port was maintained at 250°C; the detector temperature was 220°C; oven temperature was programmed as follows: 60°C for 5 min and then increased to 110°C at the rate of 5°C min^{-1}, then up to 170°C at the rate of 3°C min^{-1}, again up to 220°C at the rate of 5°C min^{-1}, at which the column was maintained for 3 min. The split ratio was 1:40 and ionization energy 70 eV.

3.1.5.2 *Analysis of non volatile pungent principles by HPLC*

Methanolic extract of dried ginger rhizomes from 12 somaclones was prepared. After preparation it shake well and kept for two hours, after 2 h it was again shaken and kept for twelve hours without disturbance. Twenty ml of supernatant was taken and it was concentrated to 2 ml on water bath and made up to 5 ml in calibrated test tube. Twenty microlitre of this sample was taken to analyse gingerol and other pungent compounds using Shimadzu HPLC with Array Detector on a C18 column (4.6 x 250mm, 5 µm). The HPLC conditions were: 280nm as detector wave length, 1 ml of flow rate and 20 microlitre volume of injection. The mobile phase was adjusted as follows: Acetonitrile : Water (65:35) with 1% acetic acid in water. Gingerol and

Dried residue after ethanol extraction

Prepared standards



Extract from somaclones after adding anthrone

Plate 4. Steps in starch estimation

related compounds were quantified in the samples by using the regression of peak areas and expressed in percentage on dry weight basis.

3.1.2 Field

The selected eight somaclones were raised in the field in an RBD design with two replications. The field was prepared by ploughing and raised beds of size 3m x1m were taken with an interchannel of 40cm width. Rhizome bits of 15- 20 g were used as seed material (Plate 1). The crop was managed as per Package of Practices, Recommendations of Kerala Agricultural University (KAU, 2011).

3.2 PREPARATION OF VALUE ADDED PRODUCTS

3.2.1 Preparation of ginger candy

Ginger candy was prepared from immature fresh ginger rhizomes (6 month old). For this, seven somaclones with variable fibre contents were selected (Plate 5). Five kg of rhizomes from selected somaclones were cleaned to remove soil particles. Cleaned rhizomes were soaked in brine solution (20% brine solution + 2500 ppm KMS + 1500 ppm Acetic acid) for 4-5 days for easy peeling and making it soft. Peeling was done manually and after peeling, rhizomes were cut in to small sized pieces. These pieces were again placed in brine solution for 8 hours. Brined ginger pieces were washed and blanched with steam at 95^0 C for 1 ½ h in Kandimat instrument. Immediately after blanching, syruping was done with sugar solution having 40^0 brix (TSS) for one hour. After one hour sugar consistency was increased to 45^0brix by adding heavy sugar syrup of 74^0brix or by evaporation and, held for one day. On second day, sugar consistency was again increased from 45 to 50^0Brix. At every four hours, this process was repeated to raise the strength of syrup from 50 to 55%, 55 to 60%, 60 to 65% and finally to 75% TSS. The entire process took 4 days to complete and on 4^{th} day sugar syrup was allowed to drip. On 5^{th} day the candy pieces were taken for drying and drying was done at 45^0C for 2 h and a gap of 30 minutes

SE 86 40

SE 86 81

SE 86 83

SE 86 131

C 86 23

C 86 139

SE HP 9

Plate 5. Selected somaclones for candy preparation

Bed preparation

Planting

Mulching

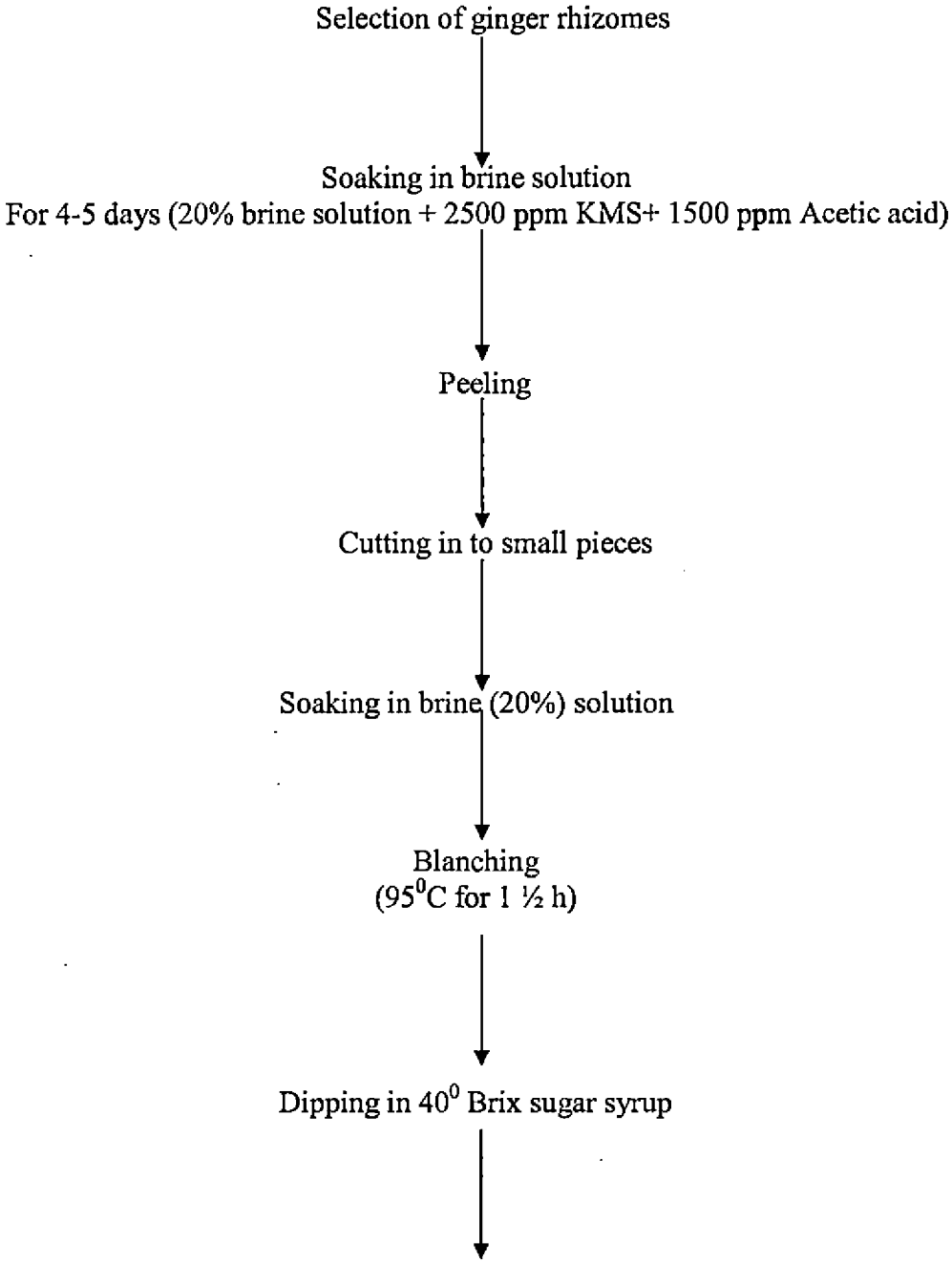
Field observation

Crop protection

Harvesting

Plate 1. Experimental field

was given. Further drying was done for one hour at 50^0C and again a gap of 30 minutes was given. Finally drying was continued at 55^0C, till the moisture content of candy reached to 8 to 10 %. The candy pieces were dusted with dextrose and it was passed through sorter to remove extra dextrose and taken for packing (Plate 6).



Peeling

Cutting

Cutting to small pieces

Soaking in brine solution

Syruping

Drying

Dried candy

Plate 6. Steps in ginger candy preparation

Raise the strength of syrup from 45% to 75% at every four hour

(4 to 5 days)

↓

Draining and drying to 8-10% moisture

(45-55^{0}C)

↓

Dusting with dextrose

↓

Packing

3.2.2 Ginger paste

Ginger paste was prepared from mature fresh ginger rhizomes (Plate 7). For this seven somaclones with variable fibre contents were selected. Five kg of selected somaclones were broken into pieces to expose the crevices and then soaked in water and then washed to remove the adhering mud. The cleaned rhizomes were scraped with a knife to remove dirt as well as spoiled portion. The rhizomes were passed through a hammer mill fitted with 30 mesh (500 mm) to get a fine paste. To this paste, vinegar and salt were added as a preservative. After adding vinegar and salt, again grinding was done, to get homogeneous paste (Plate 8).

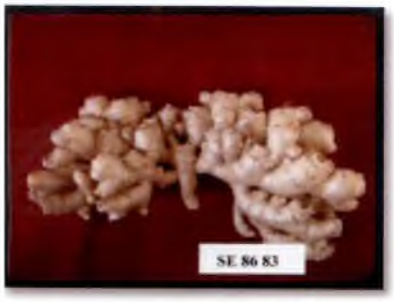
Preparation of ginger paste

Weighing of ginger

↓

C 86 123

SE HP 9



SE 86 83

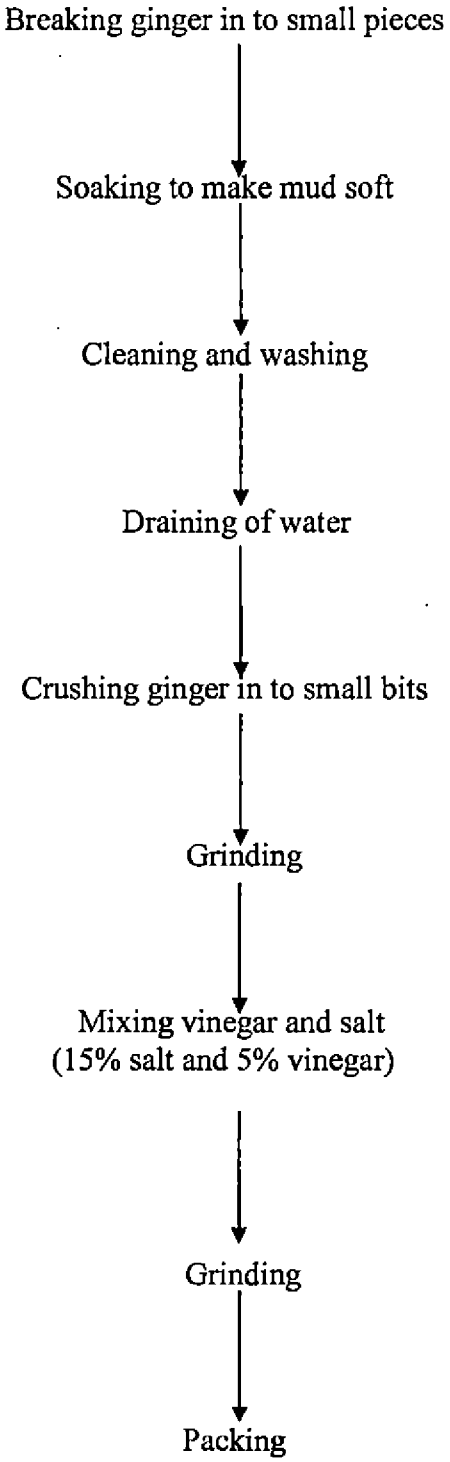
SE 86 131

SE 86 81

SE 86 40

C 86 124

Plate 7. Selected somaclones for paste preparation


Breaking ginger in to small pieces
Soaking to make mud soft
Cleaning and washing
Draining of water
Crushing ginger in to small bits
Grinding
Mixing vinegar and salt
(15% salt and 5% vinegar)
Grinding
Packing

Breaking of rhizomes

Washed rhizomes

Peeling

Grinding

Coarsely ground paste

Addition of salt

Addition of vinegar

Grinding

Fine paste

Plate 8. Steps in ginger paste preparation

3.2.3 QUALITY PARAMETER

3.2.3.1 Physical characters

3.2.3.1.1 *Easiness in peeling*

It was assessed based on peeling character of rhizomes of individual somaclones in manual peeling.

3.2.3.1.2 *Recovery of candy*

Weight of finished candy over initial weight of rhizomes was calculated and expressed as recovery percentage.

$$\text{Recovery percentage} = \frac{\text{Weight of candy after drying}}{\text{Initial weight of rhizomes}} \text{ X } 100$$

3.2.3.1.3 *Recovery of paste*

Weight of finished paste was recorded and recovery percentage was worked out.

$$\text{Recovery percentage} = \frac{\text{Weight of paste}}{\text{Initial weight of rhizomes}} \text{ X } 100$$

3.2.4 Storage stability of ginger candy and paste

The prepared candy was packed in plastic bottles and the paste was packed in three layered aluminum pouches. Both the paste and candy were stored in room temperature (26 ± 3^0C) for three months. Samples were drawn before storage and after three months to analyze the physical parameters, chemical parameters, microbial load and organoleptic quality.

3.2.5 Physical parameters

3.2.5.1 *Moisture*

Moisture content of the product was estimated by oven dry method. Ten grammes of the product was kept in hot air oven and dried to constant weight. The moisture content was calculated and expressed in percentage (Ranganna, 1995).

3.2.5.2 *Colour*

Colour of ginger candy and ginger paste was noted using the Royal Horticulture Society colour charts (Edition V).

3.2.6 Biochemical parameters

3.2.6.1 *Total Soluble Solids (TSS)*

TSS was measured by using a hand refractometer (range 0- 32^0 brix for paste and 38-92^0 brix for candy) and expressed in degree brix (^{0} Brix).

3.2.6.2 *PH*

The pH was determined in digital type pH meter.

3.2.6.3 *Titratable acidity*

The titratable acidity was determined by titrating known weight/ volume of the sample against 0.1 N NaOH solution using phenolphthalein as indicator. The acidity was calculated and expressed as per cent citric acid (AOAC, 1965).

3.2.6.4 *Non enzymatic browning*

To a ten gram sample, 100 ml of 60 per cent alcohol was added and mixed thoroughly. After keeping overnight, the contents were filtered through Whatman's filter paper. The colour was measured at 440 nm in a spectrometer using 60 per cent

alcohol as a blank. The results were reported as absorbance (optical density) value (Ranganna, 1995).

3.2.6.5 *Polyphenol oxidase (PPO) activity*

Reagents

Tris-HCL (50mM, pH 7.2) containing sorbitol (0.4M) and NaCl (10mM)
Phosphate buffer (0.1M, PH 6.5)
Catechol solution (0.01M)

The enzyme extract was prepared by homogenizing 0.5 g of ginger sample in two ml of the extraction medium containing tris HCL, sorbitol and NaCl. The homogenate was centrifuged at 2000 rpm for 10 minutes and that was used for the assay.

Assay

Phosphate buffer (2.5ml) and 0.3 ml of catechol solution were added in the cuvette and the spectrophotometer was set at 495nm. The enzyme extract (0.2ml) was added and the change in absorbance was recorded for every 30 seconds up to 5 minutes in spectrophotometer.

The activity of PPO can be calculated using the formula

Enzyme units in the sample = K x (ΔA/minute)

Where, K for catechol oxidase = 0.272

One unit of catechol oxidase is defined as the amount of enzyme that transform 1 μ mole of dihydrophenol to 1 μ mole of quinine per minute.

3.2.7 Microbiological analysis

The quantitative assay of microflora present in ginger paste and candy was carried out by serial dilution plate count method as described by Agarwal and Hasija (1986). Ten grams of sample was added to 90 ml distilled water and shaken well to

form suspension. From this, 1 ml was transferred to a test tube containing 9 ml distilled water. This gave a dilution of 10^{-2} and similarly 10^{-3}, 10^{-4} and 10^{-5}, 10^{-6} dilutions were also prepared.

Ginger paste and candy were subjected to microbiological analysis immediately after preparation and also three months after storage. The samples were analysed for the population of bacteria, mould and yeast in standard plate count. Nutrient Agar media was used for bacteria, Rose Bengal Agar media for mould and Sabouraud's Dextrose Agar media for yeast. The microbial counts were expressed in cfu/g of sample.

3.2.7.1 *Estimation of bacterial population*

Bacterial population was estimated using 10^{-5} dilution on Nutrient Agar medium. One ml of 10^{-5} dilution was pipetted in a sterile petridish using a micropipette. About 20 ml of melted and cooled Nutrient Agar (NA), media was poured in to petridish and it was swirled. After solidification, the petri plates were incubated at room temperature for 48 h. Two petridishes were kept as replicate for each sample. The bacterial colonies developed were counted and expressed as cfu/g sample.

3.2.7.2 *Estimation of mould population*

Mould was estimated using 10^{-3} dilution on, Rose Bengal agar. One ml of 10^{-3} dilution was pipette in to a sterile petridish using a micropipette. About 20 ml of melted and cooled Rose Bengal Agar media was poured in to petridish and it was swirled. After solidification, it was kept for incubation at room temperature. Two petridishes were kept as replicate for each sample. The petri plates were incubated at room temperature for 4-5 days .The mould colonies developed were counted and expressed as cfu/g sample.

3.2.7.3 *Estimation of yeast population*

Yeast population was estimated using 10^{-3} dilution on, Sabouraud's Dextrose Agar media. One ml of 10^{-3} dilution was pipetted to a sterile petridish using micropipette. About 20 ml of melted and cooled Sabouraud's Dextrose agar was poured in to petridish and it was swirled. After solidification, it was kept for incubation at room temperature. Two petridishes were kept as replicate for each sample. The petri plates were incubated at room temperature for 4-5 days .The yeast colonies developed were counted and expressed as cfu/g sample.

3.2.8 Sensory evaluation

The candy and paste prepared were evaluated for color, flavor, texture and overall acceptability by a 15 panel of testers. All the testers were briefed before evaluation. The samples were randomly coded and presented to panelists. The test panelists were asked to rate the paste and candy presented to them on a 9 point hedonic scale with the ratings of: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; and 1 = Dislike extremely. The result was analyzed by statistical software (SPSS- K related samples).

3.3.6 Statistical analysis

The evaluations for yield and quality parameters were conducted in Completely Randomized Design with 40 treatments in two replications. Data on characters studied were subjected to statistical analysis, using WASP 2.0. The data thus obtained were processed for analysis of variance. Sensory data was analyzed by statistical software (SPSS- K related samples).

RESULTS

RESULTS

The results of the investigations on "Screening somaclones of ginger (*Zingiber officinale* Rosc.) for value addition are described in this chapter under the following headings:

- ❖ Screening of somaclones for variations in quality attributes and identify novel chemotypes through chemoprofiling
- ❖ Screening somaclones for product diversification and identify elite types for value added products
- ❖ Study of storage stability of ginger candy and paste

... ..VALUATION OF SOMACLONES

4.1.1 Experimental material

Somaclones developed through indirect methods of regeneration from two induced polyploides of ginger (Z-0-78 from Himachal Pradesh treated with 0.25% colchicine by injection method and Z-0-86 from Rio-de-Jeneiro treated with 0.1% colchicine by hole method) and diploid cultivar Himachal Pradesh form the base material for the study.

Among the forty somaclones, eleven somaclones were derived from the cultivar Himachal Pradesh, twenty one somaclones were from Z-0-86 and eight somaclones were from Z-0-78.

4.1.2 Yield attributes

4.1.2.1 *Fresh rhizome yield*

The data on fresh rhizome yield per plot and per ha are presented in Table 2. The fresh rhizome yield differed significantly among the somaclones with the range of 4.61 to 15.13 kg on a per plot basis and 8.77 to 28.81 tonnes on hectare basis. Among forty somaclones, somaclone SE 86 81 showed highest per plot yield (15.13

kg m^{-1}) and highest per hectare yield (28.81 t ha^{-1}) closely followed by SE 86 131, SE 86 83 and SE 86 40. Minimum fresh rhizome yield was recorded in the somaclone SE HP 74 (4.61 kg m^{-1} and 8.77 t/ha).

Among somaclones of Z-0-86, somaclone SE 86 81 showed highest fresh rhizome yield (15.13 kg m^{-1} and 28.81 t ha^{-1}). With respect to somaclones of Z-0-78, somaclone C 78 381 recorded highest fresh rhizome yield (11.16 kg / plot) and (21.26 t/ha). Regarding somaclones of HP, somaclone CHP 282 recorded highest fresh rhizome yield (13.4 kg / plot and 25.63 t ha^{-1}). However, somaclone SE HP 74 showed lowest fresh rhizome yield (4.61 kg/plot and 8.77 t/ha).

The highest yielder among forty somaclones, somaclone SE 86 81 was on par with somaclones SE 86 131, SE 86 83, SE 86 40, C 86 23, C 86 139 and SE 86 26 with respect to fresh rhizome yield per plot and per ha. Among somaclones of Z-0-78 and HP, none of the somaclones were on par with somaclone SE 86 81.

4.1.2.2 *Dry rhizome yield*

The data on dry rhizome yield are presented in Table 2. Dry rhizome yield ranged from 1.67 to 6.43 t ha^{-1}. Among forty somaclones, somaclone C 86 23 showed highest dry rhizome yield of 6.43 t ha^{-1}, followed by somaclone SE 86 81 (6.41 t ha^{-1}). Lowest dry yield was recorded in the somaclone SEHP 73 (1.67 t ha^{-1}), followed by SE 78 12 (1.68 t ha^{-1}).

Out of twenty one somaclones of Z-0-86, somaclone C 86 23 showed highest dry yield of 6.43 t ha^{-1} and somaclone C 86 190 showed lowest dry yield of 2.48 t ha^{-1}. Among the somaclones of HP, somaclone SE HP 9 recorded highest dry yield of 5.77 t ha^{-1} and lowest was in somaclone SE HP 73 (1.67 t ha^{-1}). In general somaclones of Z-0-78, recorded lower dry rhizome yield compared to other somaclones and among these highest dry yield was recorded in the somaclone C 78 13 (4.22 t ha^{-1})

With respect to dry rhizome yield, the highest yielder C 86 23 was on par with somaclones SE 86 81, SE 86 41, SE 86 40, SE 86 83, SE HP 9, C 86 32 and SE 86 131. Among 11 somaclones of HP, somaclone SE HP 9 was on par with the somaclone C 86 23. Regarding somaclones of Z-0-78, none of somaclones were on par with the somaclone C 86 23. Compared to mean of somaclones (4.20 t ha^{-1}), out of 40 somaclones, 20 somaclones were significantly superior, five somaclones were on par and 15 somaclones had significantly lower dry yield content.

4.1.3 Quality attributes

Quality attributes such as dry recovery, volatile oil, oleoresin, starch and crude fibre contents were estimated for the forty somaclones (Table 3 and Plate 2) and the somaclones differed significantly.

4.1.3.1 *Driage*

Dry ginger recovery ranged from 15.69 to 27.13 per cent in different somaclones evaluated. Highest dry recovery was recorded in SE HP 8(27.13%), followed by somaclone SE 86 41 (25.40%) and somaclone C 86 32 (25.06%). Lowest dry recovery was recorded in somaclone SE 78 12 (15 .69%), followed by somaclone CHP 282 (16.25%).

Among somaclones of Himachal Pradesh, somaclone SE HP 8(27.13%) showed highest driage and among the twenty one somaclones of Z-0-86, somaclone SE 86 41 showed highest driage (25.40%), closely followed by somaclone C 86 32 (25.06%). Lowest driage recovery was seen in somaclone SE 86 26 (19.40%). With respect to somaclones of Z-0-78, somaclone SE 78 26 recorded maximum driage (24.40%) and lowest driage was in SE 78 12 (15.69%).

Among 40 somaclones, somaclone SE HP 8 was significantly superior and none of the somaclones were on par with SE HP 8.

Table 2. Rhizome yield of ginger somaclones

SL no:	Somaclones	Fresh rhizome yield (kg $3m^{-1}$)	Fresh rhizome yield (t ha^{-1})	Dry yield (t ha^{-1})
1	SE 86 24	7.27	13.86	2.99
2	SE 86 26	13.61	25.92	5.04
3	SE 86 40	14.44	27.50	5.86
4	SE 86 41	12.50	23.81	6.04
5	SE 86 42	12.77	24.33	5.31
6	SE 86 81	15.13	28.81	6.41
7	SE 86 83	14.51	27.64	5.66
8	SE 86 131	14.60	27.81	5.57
9	SE 86 142	9.63	18.35	3.99
10	C 86 8	11.22	21.38	4.80
11	C 86 23	13.88	26.43	6.43
12	C 86 26	12.88	24.53	5.51
13	C 86 32	12.01	22.87	5.72
14	C 86 40	13.38	25.48	5.24
15	C 86 82	9.43	17.96	3.65
16	C 86 124	11.32	21.57	5.12
17	C 86 139	13.85	26.37	5.33
18	C 86 141	7.27	13.85	2.86
19	C 86 190	5.99	11.41	2.48
20	C 86 201	7.92	15.09	3.28
21	C 86 261	13.56	25.82	5.35
22	SEHP8	9.61	18.30	4.96
23	SEHP9	12.94	24.64	5.77
24	SEHP 64	5.36	10.22	2.18
25	SEHP73	4.71	8.98	1.67
26	SEHP74	4.61	8.77	1.87
27	SEHP129	6.08	11.59	2.59
28	SEHP146	6.72	12.80	2.55
29	CHP 87	12.41	23.64	4.79
30	CHP 99	12.33	23.49	4.90
31	CHP118	11.90	22.67	4.91
32	CHP 282	13.46	25.63	4.17
33	C 78 116	4.75	9.06	1.76
34	C 78 284	11.01	20.97	3.93
35	C 78 13	11.13	21.19	4.23
36	C 78 381	11.16	21.26	4.12
37	SE 78 12	5.59	10.66	1.68
38	SE 78 26	6.88	13.09	3.20
39	SE 78 30	7.18	13.68	3.20
40	SE 78 174	7.27	14.10	2.86
Mean		10.31	19.64	4.20
CD(0.05)		4.460	8.494	2.520
CV%		21.385	21.383	22.198

4.1.3.2 *Volatile oil content*

Recovery of volatile oil varied between 1.20 to 2.40 per cent in the somaclones studied. Among forty somaclones, highest recovery of volatile oil (2.40%) was registered in the somaclone SE 86 40, followed by CHP 99 (2.30 %). Lowest volatile oil recovery (1.2%) was recorded in the somaclones SE 86 42, C 86 26, C 86 82, C 86 139, C 78 13 and SE 78 26.

Among the eleven somaclones of HP, CHP 99 and CHP 87 showed higher volatile oil recovery of 2.30 and 2.00% respectively. With respect to somaclones of Z-0-86, highest volatile oil was in somaclone SE 86 40 (2.40%), followed by somaclones C 86 40 (2.00%), C 86 190 (1.80%) and SE 86 131 (1.80%). In case of Z-0-78, somaclone C 78 284 had maximum volatile content of (2.10%), followed by somaclones SE 78 12 and SE 78 174 (2.00%).

Among 40 somaclones, somaclone SE 86 40 was on par with somaclone CHP 99 but significantly superior to other somaclones with respect to volatile oil content. Among eleven somaclones of HP, CHP 99 was on par with SE 86 40. With respect to somaclones of Z-0-86 and Z-0-78, none of the somaclones were on par with SE 86 40. Compared to mean of somaclones (1.58 %), out of forty somaclones, ten somaclones were significantly superior, thirteen were on par and seventeen were significantly lower in volatile oil content.

4.1.3.3 *Volatile oil yield per hectare*

Volatile oil yield per hectare in the somaclones ranged between 24.55 to 140.56 kg ha^{-1}. Highest volatile oil yield was recorded in the somaclone SE 86 40 (140.56 kg ha^{-1}) and lowest was in the somaclone SE HP 74 (24.55 kg ha^{-1}). Among the twenty one somaclones of Z-0-86, SE 86 40 showed highest volatile oil yield per ha (140.56 kg ha^{-1}) and C 86 141 recorded lowest volatile oil yield of 42.98 kg ha^{-1}. With respect to somaclones of HP, CHP 99 recorded highest volatile yield per hectare

(112.36 kg ha^{-1}) and lowest was in the SE HP 74 (24.55 kg ha^{-1}). In eight somaclones of Z-0-78, C 78 284 showed highest volatile oil per hectare of 81.03 t ha^{-1} and SE 78 12 showed lowest volatile yield per hectare (33.54 kg ha^{-1}).

Among 40 somaclones, SE 86 40 was significantly superior to other somaclones but on par with CHP 99. Compared to mean of somaclones (66.50 kg ha^{-1}), out of 40 somaclones 15 somaclones were significantly superior, 11 were on par and 14 had significantly lower value.

4.1.3.4 *Oleoresin content*

Oleoresin content in the somaclones ranged between 3.28 to 5.94 per cent. Highest oleoresin content was noticed in the somaclone C 86 124 (5.94 %) and lowest was noticed in the somaclone C 86 8 (3.28 %).

Almost all somaclones of Z-0-86, showed higher oleoresin recovery compared to other somaclones. Among somaclones of Z-0-86, highest oleoresin recovery was observed in somaclone C 86 124 (5.94%), followed by somaclones SE 86 41 (5.44%) and C 86 40 (5.35%). However, in case of HP, somaclone CHP 118 recorded highest oleoresin content of (5.30%) and lowest oleoresin content was in CHP 87 (3.78%).

Among 40 somaclones, somaclone C 86 124 was significantly superior and none of the somaclones were on par with C 86 124. Compared to mean of somaclones (4.53%), out of forty somaclones twenty somaclones were significantly superior, six were on par and fourteen had significantly lower oleoresin content.

4.1.3.5 *Oleoresin yield per hectare*

Oleoresin yield per hectare ranged from 63.69 to 336.64 kg ha^{-1} in the somaclones evaluated. Highest oleoresin yield was observed in the somaclone C 86 23 (336.64 kg ha^{-1}), closely followed by somaclone SE 86 81 (332.71 kg ha^{-1}). Lowest oleoresin yield was observed in the somaclone SE 78 12 (63.69 kg ha^{-1}).

Among somaclones of Z-0-86, C 86 23 recorded highest oleoresin yield (336.64 kg ha^{-1}) and C 86 190 recorded lowest oleoresin yield of 99.88 (kg ha^{-1}). Among the eleven somaclones of HP, SE HP 9 showed highest oleoresin yield of 271.73kg ha^{-1} and lowest oleoresin yield was in the SE HP 73 (77.96 kg ha^{-1}).

Among 40 somaclones, C 86 23 was significantly superior and none of the somaclones were on par with C 86 23. Compared to mean of somaclones (192.04 kg ha^{-1}), out of 40 somaclones 18 somaclones were significantly superior, seven were on par and 15 had significantly lower oleoresin yield (kg ha^{-1})

4.1.3.6 *Crude fibre content*

Crude fibre content ranged between 2.00 to 3.86 per cent. Somaclone C 86 139 showed highest fibre (3.86 %) content and lowest fibre content was observed in C 86 8 (2.00%), followed by somaclones SE HP 8 (2.1%)`and SE HP 74 (2.38%).

Among eight somaclones of Z-0-78, SE 78 174 (3.6%) and C 78 381 (3.58%) showed higher fibre content. Minimum fibre content was observed in C 78 116 (2.86%) and SE 78 12 (3.08%). With respect to somaclones of Z-0-86, C 86 139 and C 86 141 showed higher fibre content of 3.86 and 3.62 % respectively. Lowest fibre content was observed in C 86 8 (2.00%) followed by SE 86 83 (2.65%). In HP somaclones, SE HP 146 (3.52%) showed highest fibre content and SE HP 8 the lowest (2.1%).

Among forty somaclones, C 86 8 showed significantly lowest fibre but it was on par with the SE HP 8. In somaclones of Z-0-78, none of somaclones were on par with C 86 8. Among eleven somaclones of HP, SE HP 8 was on par with the C 86 8. Compared to mean of somaclones (3.07%), out of 40 somaclones 18 somaclones were significantly superior, nine were on par and 14 had significantly lower fibre content.

4.1.3.7 *Starch content*

Starch content in the somaclones ranged between 40.59 to 52.56 per cent (Table 4). Highest starch content was recorded in the somaclone SE HP 8 (52.56%) closely followed by somaclone C 86 139 (49.0%). The lowest starch content was in somaclone SE HP 74 (40.59%).

Among the somaclones of Z-0-86, C 86 139 showed higher starch content of 49.00% and lowest was in SE 86 26 (40.95 %). In case of Z-0-78, somaclones like SE 78 26 (48.92%) and SE 78 30 (46.90%) showed higher starch content. Lowest starch content was in somaclone C 78 381 (40.59%) followed by C 78 13 (40.81%) and C 78 116 (40.90%). Among the somaclones of HP, SE HP 8 recorded highest starch content (52.56%) and somaclone SE HP 74 recorded lowest starch content (40.59%).

Among 40 somaclones, SE HP 8 was significantly superior and none of the somaclones were on par with SE HP 8. Compared to mean of somaclones (44.23%), out of forty somaclones, twelve somaclones were significantly superior, ten were on par and eighteen were significantly lower in starch content.

4.1.4. Chemoprofiling of volatile oil and non volatile pungent principles

4.1.4.1 *Chemoprofiling of volatile oil*

GC-MS analysis of essential oil from dried rhizomes of 11 ginger somaclones identified a total of 44 compounds constituting 63.94 to 88.33 % of the total volatile oil constituents (Table 4). The highest percentage of essential oil constituents was identified in somaclone SE 86 131(88.33%), while the lowest was identified in C 86 124 (63.94%). The identified compounds include sesquiterpene hydrocarbons namely zingiberene, ar-curcumene, α-farnesene, germacrene D etc. Monoterpene hydrocarbons present were camphene, limonene, β-pinene, β-myrcene, β-phellandrene etc.

Table 3. Quality attributes of ginger somaclones

SL no:	Somaclones	Driage (%)	Volatile oil (%)	Volatile Oil yield (kg ha^{-1})	Oleoresin (%)	Oleoresin yield (kg ha^{-1})	Starch (%)	Crude Fibre (%)
1.	SE 86 24	21.73	1.60	47.76	4.63	137.71	41.40	2.80
2.	SE 86 26	19.40	1.40	70.55	4.53	228.62	40.95	2.75
3.	SE 86 40	21.31	2.40	140.56	4.75	278.29	41.80	3.06
4.	SE 86 41	25.40	1.40	84.55	5.44	327.15	47.48	3.25
5.	SE 86 42	21.86	1.20	63.73	5.13	272.61	41.85	3.05
6.	SE 86 81	22.26	1.60	102.56	5.19	332.71	42.95	3.00
7.	SE 86 83	20.46	1.40	78.67	3.45	195.22	42.53	2.65
8.	SE 86 131	20.06	1.80	100.29	4.29	238.76	41.72	2.85
9.	SE 86 142	21.73	1.40	55.80	4.70	187.31	42.98	2.73
10.	C 86 8	22.53	1.30	63.44	3.28	155.72	43.67	2.00
11.	C 86 23	24.26	1.60	106.38	5.23	336.64	48.20	3.31
12.	C 86 26	22.47	1.20	66.09	3.96	218.14	42.93	3.25
13.	C 86 32	25.06	1.40	80.05	4.93	282.32	46.94	2.70
14.	C 86 40	20.53	2.00	104.71	5.35	280.36	45.45	3.36
15.	C 86 82	20.26	1.20	43.78	5.25	193.99	43.65	3.32
16.	C 86 124	23.80	1.60	81.88	5.94	302.88	45.90	3.70
17.	C 86 139	20.20	1.20	63.97	4.86	259.38	49.00	3.86
18.	C 86 141	20.66	1.50	42.98	4.59	131.39	47.75	3.63
19.	C 86 190	21.75	1.80	44.57	4.03	99.88	48.51	3.30
20.	C 86 201	21.75	1.60	52.36	4.42	146.33	45.45	3.26
21.	C 86 261	20.73	1.60	85.58	3.64	195.28	45.90	3.05
22.	SEHP8	27.13	1.30	63.82	4.71	232.97	52.56	2.10
23.	SEHP9	23.40	1.60	92.32	4.71	271.73	46.58	3.01
24.	SEHP 64	21.20	1.30	29.18	4.76	103.75	41.40	2.70
25.	SEHP73	18.60	1.60	26.67	4.67	77.96	43.64	3.13
26.	SEHP74	21.33	1.30	24.55	4.51	84.37	40.59	2.38
27.	SEHP129	22.30	1.40	36.30	4.74	122.89	42.30	2.70
28.	SEHP146	19.93	1.60	40.72	3.80	96.71	43.56	3.52
29.	CHP 87	20.20	2.00	95.80	3.78	180.48	41.76	2.95
30.	CHP 99	20.90	2.30	112.36	3.93	192.34	40.86	2.75
31.	CHP118	21.60	2.00	98.22	5.30	259.13	41.76	3.20
32.	CHP 282	16.25	1.30	53.89	4.50	187.71	48.15	3.10
33.	C 78 116	19.44	1.60	28.23	4.78	84.06	40.95	2.86
34.	C 78 284	18.65	2.10	81.03	4.93	192.95	45.72	3.38
35.	C 78 13	19.90	1.20	50.69	4.24	179.35	40.81	3.56
36.	C 78 381	19.30	1.60	65.91	4.20	175.67	40.59	3.58
37.	SE 78 12	15.69	2.00	33.54	3.84	63.69	41.49	3.08
38.	SE 78 26	24.40	1.20	38.370	3.44	110.92	48.92	3.23
39.	SE 78 30	23.33	1.60	51.11	4.21	135.05	46.90	3.23
40.	SE 78 174	20.20	2.00	57.11	4.57	129.26	43.65	3.60
Mean		21.30	1.58	66.50	4.53	192.04	44.23	3.07
CD(0.05)		1.040	0.186	31.333	0.362	91.001	2.024	0.312
CV%		2.416	5.835	23.314	3.956	23.447	2.265	5.019

Sesquiterpene alcohols identified were nerolidol, α-eudesmol etc and alchohol monoterpenes such as linalool, α- terpineol etc. Monoterpenes esters such as bornyl acetate and citronellyl acetate etc and ketones aliphatic like 2- Undecanone were also present in the volatile oil. Among identified compounds, sesquiterpene hydrocarbons were major ones (70.53%), followed by monoterpenes alcohols (11.73%), monoterpenes hydrocarbons (6.03%), sesquiterpenes alcohols (5.29%), monoterpenes esters (1.20%) and miscellaneous (4.76%).

Among sesquiterpene hydrocarbons, zingiberene was the major component (23.28%) irrespective of the somaclones. The highest percentage of zingiberene was observed in the somaclone CHP 99 (29.64 %), followed by C 86 40 (28.27 %). Lowest zingiberene was observed in the somaclone C 86 124 (15.47%), followed by somaclone C 86 32 (15.50 %), but these somaclones showed higher content of ar-curcumene 11.32 and 11.05 % respectively. After zingiberene, alpha-farnesene (14.73%) was the major component and it was highest in somaclone C 86 139 (17.86%) followed by C 86 23 (17.32%). Somaclone C 86 124 showed lowest alpha-farnesene content (3.33%) among 11 somaclones. Ar-curcumene and beta-sesquiphellandrene were also detected in higher amount (8.4 and 12.97% respectively) in the somaclones. Ar-curcumene content ranged from 3.98 (SE 86 83) to 11.32 % (C 86 124) and beta-sesquiphellandrene ranged from 11.56 (C 86 124) to 14.69 % CHP 99. Among 11 somaclones, only eight somaclones showed 1, 8-cineole content and the content ranged from 0.71% (C 86 40) to 2.19 % (SE 86 83) and 1,8-cineole content was absent in C 86 139, C 86 23, C 86 124 and C 86 32. Alpha pinene, another important monoterpene observed in all the somaclones, ranged from 0.05 (C 86 32) to 1.02 % (SE 86 131). A new compound germacrene D was detected only in somaclone SE 86 83 (4.33 %) and it was absent in other somaclones. Compounds such as (+/-)-limonene, beta-citronellyl acetate, (+) aromadendren, gamma-cadinene, (+-)-beta – biasbolene, beta-phellandrene, linalool and beta-selineol were seen only in few somaclones and absent in others.

Table 4. GC-MS profile of volatile oil of ginger somaclones

COMPOUND	SE 86 81	SE 86 83	SEHP 9	SE 86 40	SE 86 131	C 86 40	CHP 99	C 86 139	C 86 23	C 86 124	C 86 32
Alpha pinene	0.85	0.96	0.76	0.55	1.02	0.06	0.27	0.14	-	0.44	0.05
(+/-)-LIMONENE	-	-	-	-	-	-	-	-	0.15	-	-
Camphene	2.37	2.59	2.15	1.71	2.60	0.29	0.90	0.52	0.52	1.50	0.14
Beta –pinene	0.11	0.12	0.09	0.07	0.12	-	-	-	-	0.06	0.56
Beta-myrcene	0.78	0.92	1.40	0.64	0.97	0.04	0.17	-	0.16	0.18	0.07
(R)-(-)-Alpha phellandrene	0.09	0.10	-	0.08	0.11	-	-	-	-	-	-
Beta phellandrene	-	2.20	-	-	-	-	-	-	-	-	-
(+) – Sabinene	1.88	-	-	1.74	2.34	0.22	0.8	0.49	0.50	0.69	0.25
Eucalyptol	-	-	-	-	-	-	-	0.73	0.63	1.46	0.76
1,8-Cineole	1.79	2.19	1.89	1.58	1.97	0.71	1.60	-	-	-	-
Alpha terpinolene	0.14	0.18	0.08	0.15	0.16	-	-	0.04	0.04	0.04	0.11
Alpha-naginatene	0.44	0.56	0.35	-	-	-	-	-	-	-	
Beta-linalool	1.12	1.22	-	1.11	1.06	0.64	0.53	0.83	0.80	0.48	0.75
(+-) – Linalool	-	-	1.13	-	-	-	-	-	-	-	-
Borneol	1.14	1.27	1.02	1.20	0.95	1.17	1.07	0.97	1.06	1.03	1.34
Alpha terpineol	0.66	0.70	0.56	0.59	0.51	0.57	0.64	0.52	0.53	0.54	0.61
Beta citronellol	2.07	0.77	0.73	0.89	0.68	0.41	0.29	-	-	0.21	0.61
R-(+)- Beta citronellol	-	-	-	-	-	-	-	0.74	0.91	-	-
Neral	0.68	1.62	1.45	1.92	1.43	0.67	0.34	0.70	0.73	-	0.61
(E)-geraniol	-	-	1.97	1.30	1.66	0.74	-	1.55	2.03	-	1.27
(E)- nerol	4.43	2.29	-	-	-	-	-	-	-	-	-
Geranial	0.94	2.34	2.11	2.77	2.13	0.94	0.54	1.16	1.18	0.32	1.15
Bornyl acetate	0.29	0.31	0.24	0.24	0.22	0.21	0.16	0.23	0.24	-	0.20
2-Undecanone	0.26	0.26	0.27	0.29	0.16	0.07	-	0.22	0.11	-	-

Beta-citronellyl acetate	0.21	-	-	-	-	-	-	-	-	-	-
Citronellyl acetate	-	-	0.19	-	0.15	-	-	0.16	0.17	-	0.11
Copaene	0.43	1.35	-	-	-	-	-	-	-	-	-
Alpha copaene	-	-	0.40	0.44	0.41	0.42	0.50	0.45	0.43	0.42	0.34
Geranyl acetate	1.04	-	1.17	0.79	0.91	0.05	-	0.85	0.98	-	0.57
Beta-elemene	0.73	0.26	0.68	0.67	0.67	0.77	0.72	0.79	0.74	0.67	0.59
(+)-Aromadendren	0.97	-	-	-	-	-	-	-	-	-	-
Allo aroma dendren	-	0.32	0.30	0.32	0.29	0.85	0.51	0.35	0.30	0.44	0.33
ar-curcumene	7.71	3.98	8.69	7.82	7.85	8.46	8.09	8.61	8.82	11.32	11.05
Germacrene D	-	4.33	-	-	-	-	-	-	-	-	--
Gamma –cadinene	-	-	-	-	-	-	-	-	-	-	2. 79
Zingiberene	22.31	23.43	23.47	24.66	24.43	28.27	29.64	24.45	24.43	15.47	15.50
Alpha-farnesene	14.89	16.17	16.36	16.51	16.84	16.27	12.71	17.86	17.32	3.33	13.77
(+) – Epibicyclo sesquiphyllandrene	-	0.54	0.55	0.57	0.58	0.81	1	0.61	0.57	8.56	-
Beta-sesquiphellandrene	12.57	11.88	13.43	14.26	13.47	15.25	15.96	15.01	14.99	12.63	13.49
(+-)-Beta – bisabolene	-	-	-	-	-	-	2.82	-	-	-	-
Elemol	0.88	0.72		0.80	0.80	1.28	1.21	1.19	1.23	1.34	1.39
+/- -Trans-nerolidol	2.57	2.39	3.06	2.79	2.73	2.74	2.64	3.24	3.16	2.64	3.69
Beta-selinenol	1.47	-	-	-	-	-	-	-	-	-	-
Beta-eudesmol	-	0.47	0.67	1.52	1.11	0.95	2	1.96	1.84	0.17	2.63
Total content	**85.82**	**86.44**	**85.17**	**87.98**	**88.33**	**82.86**	**85.11**	**84.37**	**84.57**	**63.94**	**71.94**

- **Not detected**

4.1.4.2 *Chemoprofiling of non volatile pungent principles*

The levels of gingerols and shogaols (percentage on dry weight basis of rhizomes) in the twelve ginger somaclones were determined using HPLC (Table 5). Among the gingerols and shogaols identified, 6- gingerol was the predominant one in all the ginger somaclones. Among twelve ginger somaclones, highest 6-gingerol was observed in the somaclone C 86 124 (2.44 %), followed by somaclones SE 78 26 and SE 86 81 (2.05 % and 1.89 % respectively). Lowest (6) – gingerol was observed in the somaclone C 86 139 (1.26%), followed by SE HP 9 (1.35%). Total gingerols concentration was highest in the somaclone C 86 124 (2.68%), closely followed by SE 78 26 (2.28%) and SE 86 81 (2.26%). Lowest total gingerols concentration was in the somaclone C 86 139 (1.40%) Highest level of shogaols (0.49 %) was found in the somaclone C 86 40, closely followed by SE 86 81 (0.45%) and lowest in the SE 86 42 (0.24%). Gingerol + shogaol content was highest in the somaclone C 86 124 (3.08%), closely followed by SE 86 81 (2.71%). Lowest gingerols + shogaols content was in the somaclone C 86 139(1.72%).

The yield of 6-gingerol, total gingerols, total shogaols and gingerols plus shogaols was computed from the respective contents and dry rhizome yield. Among 12 somaclones, somaclone C 86 124 showed the highest 6-gingerol yield (124.93 kg ha^{-1}) closely followed by SE 86 81 (121.15 kg ha^{-1}). Somaclone SE 78 26 showed lowest 6-gingerol yield (65. 60 kg ha^{-1}).

Total gingerols yield per hectare ranged from 74.62 to 144.87 kg ha^{-1} in the somaclones evaluated. Somaclone SE 86 81 was significantly superior (144.87 kg ha^{-1}) closely followed by C 86 124 (137.22 kg ha^{-1}). Somaclone SE 78 26 showed lowest total gingerols yield (72.96 kg ha^{-1}).

Shogaols yield per hectare ranged from 9.28 to 28.85 kg ha^{-1} in SE 78 26 and SE 86 81 respectively.

Table 5. Gingerols and shogaols content in ginger somaclones

SL no	Somaclones	Gingerols (%)				Total gingerols (%)	Total gingerols (kg ha^{-1})	Shogaols (%)	Shogaols (kg ha^{-1})	Gingerols + Shogaols (%)	Gingerols + Shogaols (kg ha^{-1})
		(6)-gingerol	(6)-gingerol (kg ha^{-1})	(8)-gingerol	(10)-gingerol						
1	SE 86 40	1.52	88.77	0.16	0.07	1.75	102.55	0.39	22.85	2.14	125.40
2	SE 86 42	1.72	91.33	0.14	0.04	1.9	100.89	0.24	12.74	2.14	113.63
3	SE 86 81	1.89	121.15	0.31	0.06	2.26	144.87	0.45	28.85	2.71	173.71
4	SE 86 83	1.53	86.60	0.16	0.06	1.75	99.05	0.34	19.24	2.09	118.29
5	SE 86 131	1.54	85.78	0.14	0.07	1.75	97.48	0.37	20.61	2.12	118.08
6	C 86 23	1.48	95.16	0.14	0.06	1.68	108.02	0.38	24.43	2.06	132.46
7	C 86 32	1.39	79.51	0.16	0.06	1.61	92.09	0.34	19.45	1.95	111.54
8	C 86 40	1.63	85.41	0.15	0.06	1.84	96.42	0.49	25.68	2.33	122.09
9	C 86 124	2.44	124.93	0.19	0.05	2.68	137.22	0.39	19.97	3.07	157.18
10	C 86 139	1.26	67.16	0.09	0.05	1.4	74.62	0.31	16.52	1.71	91.14
11	SE HP 9	1.35	77.90	0.13	0.05	1.53	88.28	0.38	21.93	1.91	110.21
12	SE 78 26	2.05	65.60	0.17	0.06	2.28	72.96	0.29	9.28	2.57	82.24

* Expressed as per cent on dry weight basis of rhizomes

With respect to gingerols plus shogaols yield, somaclone SE 86 81 showed highest value (173.71 kg ha^{-1}). Somaclone SE 78 26 showed the lowest gingerols plus shogaols yield (82.24 kg ha^{-1}).

4.1.5 Changes in quality attributes with crop maturity

The quality changes with respect to volatile oil, oleoresin, starch and crude fibre contents at 180 days and 240 days of planting was assessed (Table 6). Significant variation in quality attributes was observed with changes in maturity of rhizomes.

4.1.5.1 *Volatile oil*

As the crop matured, the volatile oil content decreased and it was highest at 180 days, irrespective of the somaclones. At full maturity, it was almost ¾th of the first harvesting (180 days). Among seven somaclones, somaclone (SE 86 40) had maximum oil content at 180 and 240 days after planting (3.45% and 2.40% respectively). Minimum oil content was observed in the somaclone C 86 139 at both harvesting stages (2.30% and 1.20% respectively).

4.1.5.2 *Oleoresin*

Oleoresin content also decreased with maturity. In all somaclones, oleoresin content was higher at 180 days of planting compared to 240 days of planting. Somaclone C 86 23 showed higher oleoresin content at both harvesting stages i.e. 180 days (5.97%) and 240 days (5.23%). Somaclone SE 86 83 showed lowest oleoresin content of 4.50% and 3.45 % at 180 days and 240 days after planting respectively, followed by somaclone SE 86 131.

4.1.5.3 *Starch*

With the advent of maturity, the starch content followed increasing trend (Table 6). In all somaclones, there was significant increase in starch content between

180 and 240 days and it was almost double at full maturity than at 180 days of planting. Somaclone C 86 139 showed maximum starch content at 240 days of planting (49.00%) and closely followed by C 86 23 (48.19%). Minimum starch content (25.07%) was observed in the somaclone SE 86 40 at 180 days whereas somaclone SE 86 131 recorded the lowest starch content at 240 days of planting (41.71%).

4.1.5.4 *Fibre*

On an average, the crude fibre content at 180 days was 2.08 % and increased to 3.11% at 240 days of harvesting. The somaclones namely C 86 139 and C 86 23 showed higher fibre content at 180 and 240 days of harvesting. It was observed that SE 86 83 had lower fibre content at both stages of harvesting (1.98 and 2.65 % respectively), followed by somaclone SE 86 131.

Table 6. Content of volatile oil, oleoresin, starch and crude fibre at different stages of maturity

SL no:	Somaclones	Volatile oil (%)		Oleoresin (%)		Starch (%)		Crude fibre (%)	
		180 Days	240 Days	180 Days	240 Days	180 Days	240 Days	180 Days	240 Days
1	C 86 23	2.55	1.60	5.97	5.23	28.45	48.19	2.10	3.31
2	C 86 139	2.30	1.20	5.65	4.86	27.51	49.00	2.35	3.86
3	SE 86 40	3.45	2.40	5.56	4.75	25.07	41.80	2.12	3.08
4	SE 86 81	2.65	1.60	5.90	5.19	25.11	42.94	2.00	3.00
5	SE 86 83	2.50	1.40	4.50	3.45	26.30	42.52	1.98	2.65
6	SE 86 131	2.75	1.80	5.05	4.29	26.26	41.71	2.00	2.85
7	SE HP 9	2.60	1.60	5.57	4.71	29.43	46.58	2.00	3.01
Mean		2.69	1.66	5.46	4.64	26.88	44.68	2.08	3.11
CD (0.05%)		0.110	0.189	0.269	0.358	1.930	2.047	0.175	0.311
CV		2.334	5.994	2.817	3.945	4.100	2.292	4.814	5.028

4.2 SCREENING SOMACLONES FOR VALUE ADDITION

The selected somaclones were screened for suitability for preparing ginger candy and paste. Seven somaclones each were selected for preparation of ginger candy (SE 86 40, SE 86 81 SE 86 83, SE 86 131, C 86 23, C 86 139 and SE HP 9) and ginger paste (SE 86 40, SE 86 81 SE 86 83, SE 86 131, C 86 23, C 86 124 and SE HP 9). The preparation of ginger candy was done at Nadukkara Agro Processing Ltd., Avoli, Ernakulam and ginger paste at M/s Manjilas Food Products, Thrissur following the procedure adopted by them.

4.2.1 Easiness in peeling

Easiness in peeling was assessed based on hand peeling. It was found that all the somaclones selected for preparation of ginger candy and paste were easy to peel except SE HP 9, which was moderately easy for peeling

4.2.2 Recovery of candy

The recovery of ginger candy from seven somaclones ranged from 62.41 % to 74.10 % (Table 7). Highest recovery (74.10%) of ginger candy was observed in somaclone SE 86 83, followed by C 86 23 (71.24%). Somaclone C 86 139 showed lowest recovery (62.41%).

4.2.3 Recovery of paste

The recovery of ginger paste from seven somaclones ranged from 105.6% to 118.8% (Table 7). Somaclone SE 86 81 had highest recovery of paste (118.8%), followed by those from somaclone C 86 124 (118.64%). Somaclone C 86 23 showed lowest recovery of paste (105.6%) compared to others.

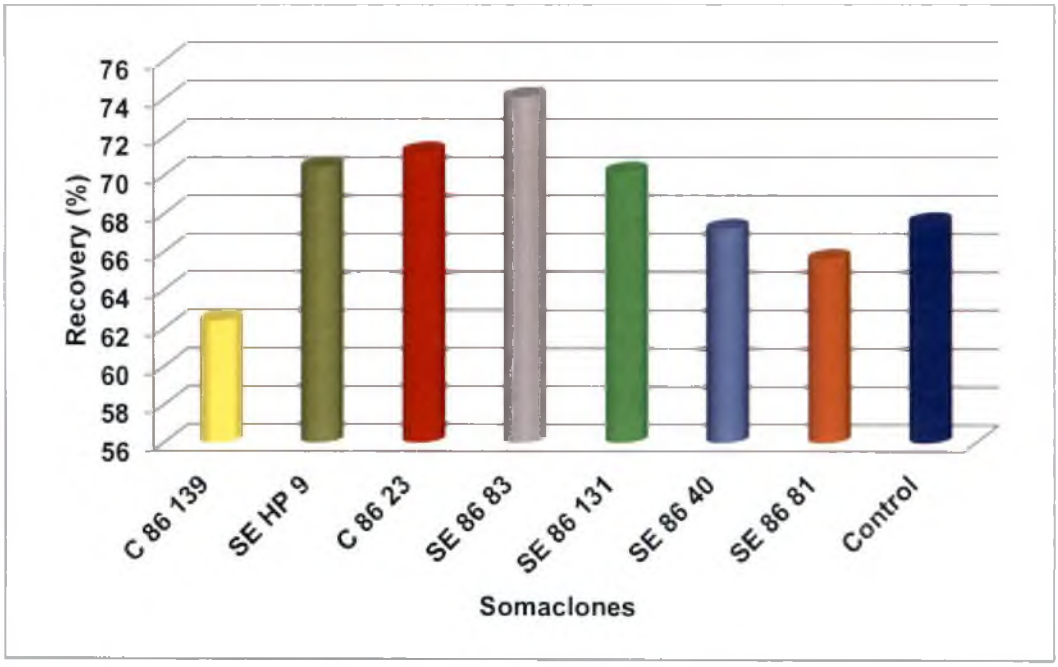


Fig 10. Recovery of ginger candy

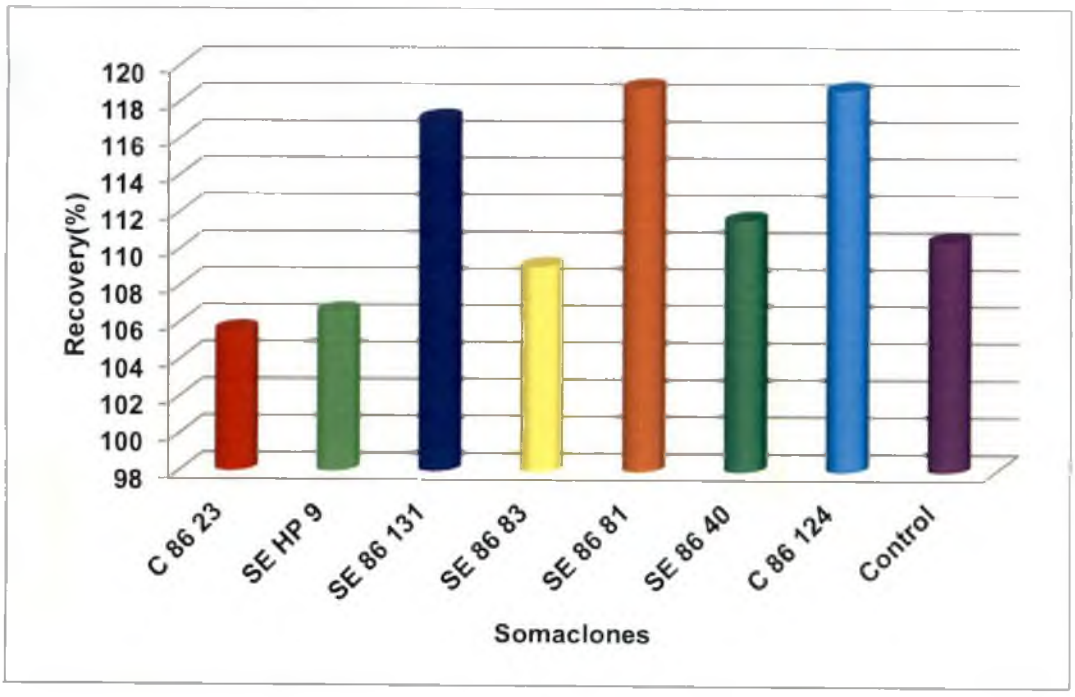


Fig 11. Recovery of ginger paste

Table 7. Recovery of ginger candy and paste

Somaclones	Recovery of candy (%)	Somaclones	Recovery of paste (%)
C 86 139	62.41	C 86 23	105.60
SE HP 9	70.43	SE HP 9	106.60
C 86 23	71.24	SE 86 131	117.10
SE 86 83	74.10	SE 86 83	109.10
SE 86 131	70.19	SE 86 81	118.80
SE 86 40	67.22	SE 86 40	111.58
SE 86 81	65.62	C 86 124	118.64
Control	67.56	Control	110.50
CD (0.05%)	1.513	CD (0.05%)	1.486
CV%	1.274	CV%	1.296

4.3 STABILITY OF QUALITY PARAMETERS OF GINGER CANDY DURING STORAGE

The prepared ginger candy and paste were packed in plastic bottles and three layered aluminum pouches respectively and stored under ambient conditions for three months. The physico-chemical characteristics and microbial load in the products were recorded immediately after preparation of products and after three months of storage.

4.3.1 Physical characteristics

4.3.1.1 *Moisture content*

Initial moisture content of ginger candy was observed between 9.89 to 11.11%, with somaclone SE HP 9 recording highest value (11.11%). Minimum moisture (9.89%) was observed in somaclones C 86 139, SE 86 83, SE 86 131 and control.

Moisture content of ginger candy from all somaclones and control showed a decreasing trend during storage (Table 8). Somaclone SE 86 81 showed maximum moisture loss (8.22%) after three months of storage. Minimum moisture loss was recorded in somaclone SE HP 9 (4.60 %) after three months of storage.

4.3.1.2 *Colour*

Colour of ginger candy was assessed by using the Royal Horticulture Society Colour Charts (Edition V). Initially all the somaclones showed the same colour of light yellow (18b).

Compared to initial colour, there was slight colour change in ginger candy from the somaclones after three months of storage. Ginger candy from somaclones C 86 139, SE HP 9 and C 86 23 showed light yellow colour (20B), whereas somaclones SE 86 83, SE 86 131, SE 86 40, SE 86 81 and control showed light yellow colour (18A).

4.3.2 Biochemical parameters

4.3.2.1 *TSS (^{0}B)*

Initial TSS content of ginger candy was observed between 68.5 to 69 ^{0}B. Ginger candy from all the somaclones except C 86 139 were on par with respect to TSS content.

TSS content of ginger candy showed increasing trend during storage (Table 9a). Ginger candy from somaclone SE 86 83 showed highest TSS content (69.44 ^{0}B) after 3 months of storage, followed by SE 86 81 and control (69.40 ^{0}B). Minimum TSS content was observed in somaclone C 86 139, which had minimum TSS at initial and after 3 months of storage also (68.50 and 68.92 ^{0}B respectively).

4.3.2.2 *pH*

Initial pH content of ginger candy from different somaclones was observed between 3.51 to 3.60, with the maximum in somaclone SE 86 131 and minimum in somaclones SE 86 83 and SE 86 40.

On storage all somaclones including control showed decreasing trend in pH (Table 9a). After three months of storage maximum pH (3.57) was in somaclone SE 86 131 followed by SE HP 9 (3.55) and minimum pH (3.48) was in somaclone SE 86 83.

Table 8. Effect of storage on physical characteristics of ginger candy

	Moisture (%)			Colour	
Somaclones	**Initial**	**3 MAS**	**% loss on storage**	**Initial**	**3 MAS**
C 86 139	9.89	9.35	5.46	light yellow (18b)	light yellow (20B)
SE HP 9	11.11	10.60	4.60	light yellow (18b)	light yellow(20B)
C 86 23	10.07	9.55	5.16	light yellow (18b)	light yellow (20B)
SE 86 83	9.89	9.38	5.16	light yellow (18b)	light yellow (18A)
SE 86 131	9.89	9.36	5.36	light yellow (18b)	light yellow (18A)
SE 86 40	10.13	9.60	5.24	light yellow (18b)	light yellow (18A)
SE 86 81	10.22	9.38	8.22	light yellow (18b)	light yellow (18A)
Control	9.89	9.36	5.36	light yellow (18b)	light yellow (18A)
CD (0.05%)	0.282	0.397	-	-	-
CV%	1.604	2.394	-	-	-

4.3.2.3 *Titratable acidity*

In ginger candy, initial titratable acidity recorded among the somaclones ranged between 0.16 to 0.19%. The candy from somaclones C 86 139, C 86 23, SE 86 83, SE 86 40, SE 86 81 and control showed maximum acidity (0.19%) and it was significantly superior to somaclones SE HP 9 and SE 86 131 which showed minimum acidity (0.16%).

The titratable acidity in ginger candy showed increasing trend during storage in the all somaclones and control (Table 9a). Candy from somaclones SE HP 9 and SE 86 131 had minimum (0.19%) titratable acidity at end the of storage period. Somaclones C 86 139 and SE 86 81 showed maximum (0.26%) titratable acidity after

storage. Titratable acidity of somaclone SE 86 40 was 0.25 % and same value was observed in somaclone C 86 23, SE 86 83 and control.

4.3.2.4 *Non enzymatic browning*

Initial non enzymatic browning in ginger candy ranged between 0.090 to 0.125. In ginger candy, highest non enzymatic browning was observed in somaclone C 86 139 (0.125), followed by somaclone SE HP 9 (0.119) (Table 9b). Lowest non enzymatic browning was in somaclone SE 86 131 (0.09), followed by somaclone SE 86 81 (0.101). Somaclones SE HP 9 (0.119), C 86 23 (0.115), SE 86 40 (0.11) were on par with control (0.115).

Non enzymatic browning increased in ginger candy during storage (Table 10b). Somaclone C 86 139 showed maximum (0.150) non enzymatic browning after 3 months of storage and it was significantly higher than that of somaclone SE 86 131, which had minimum value (0.113) after 3 months of storage.

4.3.2.5 *Polyphenol oxidase activity*

No polyphenol oxidase activity was detected in any of the candy from different somaclones (Table 9b).

Polyphenol oxidase activity was observed in all the candy from different somaclones of ginger candy after three months of storage (Table 9b). The highest PPO activity was observed in candy from somaclone C 86 23 (0.25 units/litre), followed by somaclone SE 86 81 (0.22 units/liter) and lowest PPO activity was in SE 86 131 (0.08 units/litre).

Table 9a. Effect of storage on biochemical characteristics of ginger candy

	TSS(^{0}B)		pH		Titratable acidity (%)	
Somaclones	**Initial**	**3 MAS**	**Initial**	**3 MAS**	**Initial**	**3 MAS**
C 86 139	68.50	68.92	3.53	3.50	0.19	0.26
SEHP9	69.00	69.38	3.57	3.55	0.16	0.19
C 86 23	68.75	69.17	3.52	3.49	0.19	0.25
SE 86 83	69.00	69.44	3.51	3.48	0.19	0.25
SE 86 131	69.00	69.39	3.60	3.57	0.16	0.19
SE 86 40	69.00	69.36	3.51	3.49	0.19	0.25
SE 86 81	69.00	69.40	3.53	3.50	0.19	0.26
Control	69.00	69.40	3.55	3.52	0.19	0.25
CD (0.05%)	0.037	0.046	0.024	0.021	0.006	0.006
CV%	0.031	0.039	0.399	0.349	1.903	1.449

Table 9b. Effect of storage on biochemical characteristics of ginger candy

	Non enzymatic browning (absorbance)		Polyphenol oxidase activity (units / litre)	
Somaclones	**Initial**	**3 MAS**	Initial	3 MAS
C 86 139	0.125	0.150	ND	0.20
SEHP9	0.119	0.142	ND	0.18
C 86 23	0.115	0.141	ND	0.25
SE 86 83	0.107	0.131	ND	0.10
SE 86 131	0.090	0.113	ND	0.08
SE 86 40	0.111	0.129	ND	0.14
SE 86 81	0.101	0.124	ND	0.22
Control	0.115	0.139	ND	0.13
CD (0.05%)	0.003	0.002	-	0.0118
CV%	1.371	1.058	-	2.582

4.3.3 Enumeration of microbial flora

4.3.3.1 *Bacterial population*

No bacterial growth was observed in the ginger candy from different somaclones and control immediately after preparation. However, bacterial growth was detected in all somaclones of ginger candy (2.0-5.0 $X10^{-5}$) after three months of storage (Table 10 and Plate 9). In ginger candy, somaclone SE HP 9 showed highest bacterial population of 5.0 X 10^{-5}. However, somaclones SE 86 131, SE 86 40 and control showed the lowest bacterial population of 2.0 X 10^{-5}, after three months of storage.

4.3.3.2 *Mould population*

In ginger candy, mould population was not detected in any of the somaclones immediately after preparation, however it was found in some somaclones of ginger candy (1.0-2.0 X 10^{-3}), after three months of storage (Table 10 and Plate 9). Candy prepared from somaclones SE HP 9 and SE 86 81 showed higher mould population of 2.0 X 10^{-3}, followed by those from somaclones C 86 139 and SE 86 83 (1.0 X 10^{-3}). Other somaclones did not show any mould growth.

4.3.3.4 *Yeast population*

Yeast population was not detected in any of the candy from different somaclones initially, however it was detected in ginger candy from some of the somaclones after three months of storage (Table 10 and Plate 9). Ginger candy from somaclone SE HP 9 showed highest yeast population (2.0 X 10^{-3}), followed by somaclones C 86 139, SE 86 81 and SE 86 83 and (1.0 X 10^{-3}), whereas in others no yeast growth was noted even after 3 months of storage.

Bacteria

Mould

Yeast

Initial microbial count

Bacteria

Mould

Yeast

Microbial count after three months of storage

Plate 9. Microbial count in ginger candy

Table 10. Effect of storage on microbial population of ginger candy

Somaclones	Initial (cfu/g)			3 MAS (cfu/g)		
	Bacteria x 10^{-5}	Yeast x 10^{-3}	Fungi x 10^{-3}	Bacteria x 10^{-5}	Yeast x 10^{-3}	Fungi x 10^{-3}
C 86 139	ND	ND	ND	3	1	1
SEHP9	ND	ND	ND	5	2	2
C 86 23	ND	ND	ND	3	ND	ND
SE 86 83	ND	ND	ND	3	1	1
SE 86 131	ND	ND	ND	2	ND	ND
SE 86 40	ND	ND	ND	2	ND	ND
SE 86 81	ND	ND	ND	3	1	2
Control	ND	ND	ND	2	ND	ND

(ND- Not Detected and 3 MAS- Months after storage)

4.3.4 Sensory evaluation

The prepared candy was subjected to sensory evaluation by a panel of 15 judges using nine point hedonic scale and the best somaclones for ginger candy preparation were identified based on the sensory scores (Table 11a). Ginger candy, from somaclone SE 86 40 scored the highest total score (65.39) and it was on par with control (64.98) and SE 86 131 (60.03). Candy from somaclone SE 86 83 scored the lowest total score (45.61) and it was identified as the least preferred sample and other acceptable somaclones for candy were SE 86 81 and SE HP 9.

Sensory scores of ginger candy showed a decreasing trend during storage (Table 11b). After three months of storage, ginger candy from somaclone SE 86 40 showed significantly higher score (64.34) followed by control (63.93) and minimum score was in somaclone SE 86 83 (44.56). Candy from somaclones SE 86 40, SE 86 131, SE HP 9 and control were under acceptable limits based on the scores, after three months of storage.

Table 11a. Sensory scores of fresh ginger candy

Samples	Appearance	Colour	Flavor	Texture	Odour	Taste	After taste	Overall acceptability	Total score
C 86 139	6.77	5.70	5.93	5.63	5.37	5.87	5.73	6.47	47.47
SE HP 9	6.77	5.90	5.47	5.87	5.87	7.43	7.77	6.47	51.55
C 86 23	6.43	5.23	5.90	5.50	5.40	5.20	6.17	6.60	46.43
SE 86 83	6.37	5.97	4.77	5.87	5.87	4.63	6.4	5.73	45.61
SE 86 131	6.43	7.63	7.27	7.43	7.77	7.5	8.43	7.57	60.03
SE 86 40	8.83	7.90	7.60	8.00	8.17	7.93	8.93	8.03	65.39
SE 86 81	7.30	6.03	6.23	5.37	6.23	6.33	7.03	5.90	50.42
Control	8.53	7.63	7.83	8.23	8.33	8.10	8.10	8.23	64.98
Kendal's W test	0.612**	0.338**	0.224**	0.317**	0.432**	0.312**	0.431**	0.330**	

** Significant at 1% level

Table 11b. Sensory scores of ginger candy after three months of storage

Samples	Appearance	Colour	Flavor	Texture	Odour	Taste	After taste	Overall acceptability	Total score
C 86 139	6.62	5.55	5.83	5.53	5.27	5.72	5.58	6.32	46.42
SE HP 9	6.62	5.75	5.37	5.77	5.77	7.28	7.62	6.32	50.50
C 86 23	6.28	5.08	5.80	5.40	5.30	5.05	6.02	6.45	45.38
SE 86 83	6.22	5.82	4.67	5.77	5.77	4.48	6.25	5.58	44.56
SE 86 131	6.28	7.48	7.17	7.33	7.67	7.35	8.28	7.42	58.98
SE 86 40	8.68	7.75	7.50	7.90	8.07	7.78	8.78	7.88	64.34
SE 86 81	7.15	5.88	6.13	5.27	6.13	6.18	6.88	5.75	49.37
Control	8.38	7.48	7.73	8.13	8.23	7.95	7.95	8.08	63.93
Kendal's W test	0.606**	0.332**	0.217**	0.309**	0.426**	0.306**	0.423**	0.320**	

4.4 STABILITY OF QUALITY PARAMETERS OF GINGER PASTE DURING STORAGE

4.4.1 Physical characteristics

4.4.1.1 *Moisture content*

The moisture content of the ginger paste was recorded immediately after preparation and after three months of storage. Initially, highest moisture content was observed in ginger paste from somaclone SE HP 9 (67.41%), followed by control (66.80%). Ginger paste from somaclone C 86 23 showed minimum moisture content of 65.10%, followed by paste from SE 86 131 and C 86 124 (66.16 and 66.25% respectively).

Moisture content of ginger paste from all the somaclones and control showed a decreasing trend during storage (Table 12 and Fig 13). Somaclone SE 86 131 recorded minimum moisture loss of (0.44%), followed by somaclone C 86 23 (0.46%). Maximum moisture loss was in somaclone C 86 124 (0.56%).

4.4.1.2 *Colour*

Colour of ginger paste was assessed by using the Royal Horticulture Society Colour Charts (Edition V). In ginger paste, all somaclones exhibited pale yellow colour (12D), except somaclone SE 86 83, which had pale creamy yellow colour (13B).

There was no notable colour change in ginger paste from different somaclones during storage.

Table 12. Effect of storage on physical characteristics of paste

Somaclones	Moisture (%)			Colour	
	Initial	3 MAS	% loss on storage	Initial	3 MAS
C 86 23	65.10	64.80	0.46	pale yellow colour (12D)	pale yellow colour (12D)
SE HP 9	67.41	67.04	0.55	pale yellow colour (12D)	pale yellow colour (12D)
SE 86 131	66.16	65.87	0.44	pale yellow colour (12D)	pale yellow colour (12D)
SE 86 83	66.75	66.41	0.51	creamy yellow colour (13B)	creamy yellow colour (13B)
SE 86 81	66.75	66.40	0.52	pale yellow colour (12D)	pale yellow colour (12D)
SE 86 40	66.67	66.32	0.52	pale yellow colour (12D)	pale yellow colour (12D)
C 86 124	66.25	65.88	0.56	pale yellow colour (12D)	pale yellow colour (12D)
Control	66.80	66.44	0.54	pale yellow colour (12D)	pale yellow colour (12D)
CD (0.05%)	0.289	0.337	-	-	-
CV%	0.250	0.295	-	-	-

4.4.2 Biochemical parameters

4.4.2.1 *Total Soluble Solids*

Initial TSS content of ginger paste was observed between 24.27 to 25.75 ^{0}B. Somaclone SE 86 81 had maximum TSS content (25.75 ^{0}B) and it was on par with that of control (25.67^{0}B). Lowest TSS was observed in somaclone SE 86 131 (24.27^{0}B), followed by somaclone C 86 23 (24.30^{0}B).

TSS content of ginger paste showed increasing trend during storage, irrespective of the somaclones (Table 13a). Somaclone SE 86 40 recorded maximum TSS (25.99 ^{0}B) after 3 months of storage, followed by SE 86 81 (25.96 ^{0}B). Somaclone SE 86 131 showed minimum TSS (24.48 ^{0}B) after storage.

4.4.2.2 *pH*

Initial pH content of ginger paste from different somaclones was observed between 3.62 to 3.75. Somaclone SE 86 81 showed maximum pH (3.75) which was

on par with that of control (3.73) and minimum pH was noticed in the somaclone SE 86 131 (3.62), followed by somaclone C 86 23 (3.63).

pH content of ginger paste showed decreasing trend during storage, irrespective of the somaclones (Table 13a). Somaclone SE 86 81 recorded highest pH (3.73) content in ginger paste and it was on par with that of control (3.71), after 3 months of storage. Minimum pH (3.61) was observed in somaclone SE 86 131, which was on par with that of somaclone C 86 23 (3.62).

4.4.2.3 *Titratable acidity*

In ginger paste, initial titratable acidity recorded among the somaclones ranged between 3.30 to 3.75%. Somaclone SE 86 131 recorded the highest acidity (3.75). However somaclones SE HP 9, SE 86 81 and control showed minimum acidity (3.30%).

Titratable acidity in ginger paste showed increasing trend in during storage in all somaclones and control (Table 13a). Paste from somaclone SE 86 131showed highest titratable acidity (3.95%), after 3 months storage. Minimum titratable acidity was in control (3.46%) and it was on par with that of somaclone SE 86 81 (3.47 %), after 3 months of storage

4.4.2.4 *Non enzymatic browning*

Initially, non enzymatic browning in ginger paste from different somaclones ranged between 0.129 to 0.160. Maximum non enzymatic browning (0.160) was observed in the somaclone C 86 23, followed by somaclone SE 86 83 (0.159), while the somaclone SE 86 131 had the minimum non enzymatic browning (0.129). Somaclones SE 86 40 (0.140) and C 86 124 (0.145) were on par with that of control (0.140).

Non enzymatic browning increased in ginger paste during storage (Table 13b). Initially paste from somaclone C 86 23 showed highest (0.160) non enzymatic browning but after three months of storage, somaclone SE 86 83 had highest (0.181) non enzymatic browning. Minimum non enzymatic browning (0.140) was in paste from somaclone SE 86 131, followed by control (0.150) after 3 months of storage.

4.4.2.5 *Polyphenol oxidase activity*

Polyphenol oxidase activity was not detected in ginger paste from any of the somaclones (Table 13b), but it was observed in all the pastes after three months of storage. Paste from somaclone C 86 23 (0.98 units/litre) showed highest polyphenol oxidase activity, followed by those from SE HP 9 (0.55 units/litre). Paste from somaclone SE 86 131 showed lowest PPO activity (0.25 units/litre) after three months of storage.

4.4.2.6 Enumeration of microbial flora

4.4.2.6.1 *Bacterial population*

Initially no bacterial growth was observed in ginger paste from any of the somaclones. However, bacterial growth was detected in ginger paste from all the somaclones and control (1.0-2.0 $X10^{-5}$) after three months of storage (Table 14 and Plate 10). Ginger paste from all somaclones and control showed bacterial population of 2.0 X 10^{-5} except somaclone SE 86 131 and somaclone SE 86 40 which showed lower bacterial population of 1.0 X 10^{-5}, after three months of storage.

4.4.2.6.2 *Mould population*

In ginger paste, mould population was not detected in any of the somaclones immediately after preparation, however it was found in some ginger paste from somaclones (1.0 $X10^{-3}$), after 3 months of storage (Table 14 and Plate 10).

Table 13a. Effect of storage on biochemical characteristics of ginger paste

	TSS(^{0}B)		pH		Titratable acidity (%)	
Somaclones	**Initial**	**3 MAS**	**Initial**	**3 MAS**	**Initial**	**3 MAS**
C 86 23	24.31	24.56	3.63	3.62	3.69	3.82
SE HP 9	25.63	25.89	3.72	3.70	3.30	3.49
SE 86 131	24.27	24.48	3.62	3.61	3.75	3.90
SE 86 83	24.43	24.67	3.70	3.69	3.37	3.50
SE 86 81	25.75	25.96	3.75	3.73	3.30	3.47
SE 86 40	24.78	24.99	3.67	3.65	3.37	3.50
C 86 124	25.43	25.70	3.70	3.68	3.37	3.53
Control	25.67	25.94	3.73	3.71	3.30	3.46
CD (0.05%)	0.054	0.025	0.045	0.048	0.065	0.062
CV%	0.124	0.056	0.698	0.75	1.097	1.003

Table 13b. Effect of storage on biochemical characteristics of ginger paste

	Non enzymatic browning (absorbance)		Polyphenol oxidase activity (units / litre)	
Somaclones	**Initial**	**3 MAS**	Initial	3 MAS
C 86 23	0.160	0.170	ND	0.98
SE HP 9	0.130	0.150	ND	0.55
SE 86 131	0.129	0.145	ND	0.25
SE 86 83	0.159	0.181	ND	0.45
SE 86 81	0.135	0.155	ND	0.40
SE 86 40	0.140	0.162	ND	0.50
C 86 124	0.145	0.166	ND	0.50
Control	0.140	0.150	ND	0.46
CD (0.05%)	0.006	0.006	-	0.017
CV%	2.564	2.273	-	1.874

(ND- Not detected and 3 MAS- Months after storage)

Ginger paste from three somaclones (SE HP 9, SE 86 81 and C 86 124) showed mould population of 1.0 X 10^{-3} after 3 months of storage. However mould growth was not seen in other somaclones even after 3 months of storage.

4.4.2.6.3 *Yeast population*

Yeast population was not detected in any of the paste from different somaclones at initial stage, however it was detected in some samples after 3 months of storage (Table 14 and Plate 10). Ginger paste from somaclones SE HP 9 and C 86 124 alone showed yeast population of 1.0 x 10^{-3}, after 3 months of storage.

Table 14. Effect of storage on microbial population of ginger paste

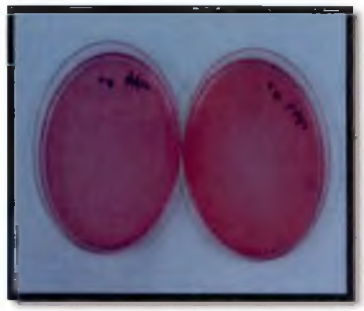
Somaclones	Initial (cfu/g)			3 MAS (cfu/g)		
	Bacteria x 10^{-5}	Yeast x 10^{-3}	Fungi x 10^{-3}	Bacteria x 10^{-5}	Yeast x 10^{-3}	Fungi x 10^{-3}
C 86 23	ND	ND	ND	2	ND	ND
SE HP 9	ND	ND	ND	2	1	1
SE 86 131	ND	ND	ND	1	ND	ND
SE 86 83	ND	ND	ND	2	ND	ND
SE 86 81	ND	ND	ND	2	ND	1
SE 86 40	ND	ND	ND	1	ND	ND
C 86 124	ND	ND	ND	2	1	1
Control	ND	ND	ND	2	ND	ND

(ND- Not detected and 3 MAS- Months after storage)

4.4.2.6.4 Sensory evaluation

The prepared paste was subjected to sensory evaluation by a panel of 15 judges using nine point hedonic scale and the best somaclones for ginger paste preparation were identified based on the sensory scores. (Table 15a). Ginger paste, somaclone SE 86 131 was identified as the best sample as its score for color, flavor, texture, overall acceptability and most importantly total score was highest (66.4) compared to the others, followed by somaclone C 86 23 (64.35). Paste from

Bacteria

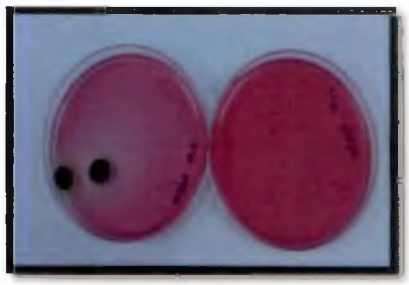


Mould

Yeast

Initial microbial load

Bacteria



Mould

Yeast

Microbial load after three months of storage

Plate 10. Microbial load in ginger paste

somaclone C 86 124 was considered the least preferred sample because its score for color, flavor, texture and overall acceptability was lowest (39) and other acceptable somaclones for paste were SE 86 40, SE 86 83 and SE HP 9.

Sensory score of ginger paste showed a declining trend during storage, irrespective of somaclones (Table 15b). In ginger paste, SE 86 131 had maximum total score (65.35) after 3 months of storage, followed by C 86 23 (63.3) and minimum total score was recorded in somaclone C 86 124 (37.95), followed by SE 86 81 (44.35). Paste from somaclones SE 86 131, C 86 23, SE HP 9 and SE 86 83 were under acceptable limits based on the scores, after three months of storage.

Table 15a. Sensory scores of fresh ginger paste

Somaclones	Appearance	Colour	Flavor	Texture	Odour	Taste	After taste	Overall acceptability	Total score
C 86 23	8.00	8.10	7.90	7.60	8.05	8.00	8.60	8.10	64.35
SE HP 9	7.45	6.25	5.65	6.30	5.50	6.65	6.80	6.70	51.3
SE 86 131	8.05	8.35	8.15	7.70	8.65	8.35	8.70	8.45	66.4
SE 86 83	6.50	6.06	6.00	5.85	7.10	7.70	6.40	6.50	52.11
SE 86 81	5.85	5.50	5.90	5.25	6.00	5.65	5.55	5.70	45.4
SE 86 40	5.05	5.50	6.60	7.90	5.90	5.90	6.20	7.00	50.05
C 86 124	4.40	4.75	5.45	4.75	5.25	5.30	4.20	4.90	39
Control	6.70	7.70	6.55	6.35	5.65	5.45	5.55	5.30	49.25
Kendal's W test	0.515**	0.406**	0.353**	0.332**	0.403**	0.387**	0.463**	0.459**	

** Significant at 1% level

Table 15b. Sensory scores of ginger paste after three months of storage

Somaclones	Appearance	Colour	Flavor	Texture	Odour	Taste	After taste	Overall acceptability	Total score
C 86 23	7.85	7.95	7.8	7.5	7.95	7.85	8.45	7.95	63.3
SE HP 9	7.3	6.1	5.55	6.2	5.4	6.5	6.65	6.55	50.25
SE 86 131	7.9	8.2	8.05	7.6	8.55	8.2	8.55	8.3	65.35
SE 86 83	6.35	5.91	5.9	5.75	7	7.55	6.25	6.35	51.06
SE 86 81	5.7	5.35	5.8	5.15	5.9	5.5	5.4	5.55	44.35
SE 86 40	4.9	5.35	6.5	7.8	5.8	5.75	6.05	6.85	49
C 86 124	4.25	4.6	5.35	4.65	5.15	5.15	4.05	4.75	37.95
Control	6.55	7.55	6.45	6.25	5.55	5.3	5.4	5.15	48.2
Kendal's W test	0.507**	0.398**	0.345**	0.326**	0.396**	0.380**	0.455**	0.450**	

DISCUSSION

DISCUSSION

Ginger is one of the most widely cultivated and used spice around the globe. India is a major ginger growing country contributing 35 per cent of global production. Kerala has a prominent position as a ginger growing state and produces Cochin and Calicut ginger renowned for their intrinsic qualities. In spite of these, significant strides could not made in the processing sector unlike small growers like Australia and Fiji which are the major exporters of value added products from ginger. This is primarily due to lack of varieties having good processing qualities. Somaclonal variation act as a major source of variability for crop improvement.

Induction of variability through induced polyploidy attempted at Department of Plantation Crops & Spices, College Of Horticulture, Vellanikkara, has succeeded in the development of two autotetraploids (Sheeba, 1996) with desirable quality attributes like low fibre content and high aromatic oil and oleoresin but susceptible to the diseases which restricts their commercial utility (Shankar, 2003). In order to increase the spectrum of variability in these tetraploids, induction of variation *in vitro* through indirect methods of regeneration and mutagenesis was attempted as part of DBT funded project from 2006 to 2010. This has resulted in development of potential variants which on preliminary evaluation revealed wide variability in quality Kurian (2010) and Dev (2013).

The present study entitled "Screening somaclones of ginger for value addition (*Zingiber officinale* Rosc.)" was taken up in this background at College of Horticulture, Vellanikkara during 2013-15 to evaluate forty somaclones for quality attributes and value addition. These forty somaclones were selected from a base population of 289 somaclones, developed through indirect organogenesis and indirect embryogenesis with and without mutagenesis from three cultivars (two induced polyploids Z-0-78, Z-0-86 and diploid cultivar Himachal Pradesh), at the Department of Plantation Crops & Spices, College Of Horticulture, Vellanikkara. Out of 40

somaclones, five somaclones namely SE 86 41, C 86 201, C 86 261, CHP 87 and C 78 116 were *in vitro* induced mutants with 10 Gy gamma rays.

5.1 EVALUATION OF SOMACLONES

5.1.1 Experimental material

The somaclones derived through indirect methods of regeneration with and without irradiation from two induced polyploids (Z-0-78 and Z-0-86) and a diploid cultivar Himachal Pradesh form the base material for the study. Among the forty somaclone, eleven somaclones were from HP, twenty one somaclones were derived from Z-0-86 and eight somaclones from Z-0-78.

5.1.2 Fresh and dry rhizome yield

Significant difference was observed in somaclones with respect to fresh and dry rhizome yield (Table 2). The fresh yield of rhizome ranged between 8.77 to 28.81 t ha^{-1} and dry rhizome yield from 1.67 to 6.43 t ha^{-1} in the somaclones. Among forty somaclones, somaclone SE 86 81 showed highest per plot yield (15.13 kg/plot) and highest per hectare yield (28.81t/ha) followed by SE 86 131, SE 86 83, SE 86 40 and C 86 8. Minimum per plot and minimum per ha yield was recorded in the somaclone SE HP 73 (4.61 kg/plot and 15.13 t/ha respectively). Somaclone C 86 23 showed highest dry yield of 6.43 t ha^{-1}, followed by somaclone SE 86 81(6.41 t ha^{-1}). Lowest dry yield was recorded in the somaclone SE HP 73 (1.67 t ha^{-1}), followed by somaclone SE 78 12 (1.68 t ha^{-1}). Resmi and Shylaja (2012) reported that 30 per cent somaclones were found superior to conventionally propagated plants for rhizome characters and eighteen per cent somaclones exhibited superiority in yield over conventionally propagated plants giving a yield increase of 13 per cent. Samsudeen (1996), Shylaja *et al.* (2003), Kurian (2010) and Shylaja *et al.* (2010) also reported variability in rhizome yield in ginger somaclones.

Somaclones derived from polyploid parent Z-0-86 recorded higher fresh and dry rhizome yield compared to somaclones derived from Z-0-78 and HP (Table 16). Fresh rhizome yield per hectare varied from 11.41 t ha^{-1} to 28.81 t ha^{-1} in somaclones of Z-0-86, 8.77 to 25.63 in somaclones of HP, 9.06 t ha^{-1} to 21.26 t ha^{-1} in somaclones of Z-0-78. Dry rhizome yield per hectare varied from 2.48 t ha^{-1} to 6.43 t ha^{-1} in somaclones of Z-0-86, 1.67 to 5.77 t ha^{-1} in somaclones of HP and 1.68 to 4.23 t ha^{-1} in somaclones of Z-0-78. Somaclones of Z-0-86 showed highest mean fresh rhizome yield (22.42 t ha^{-1}) compared to somaclones of Z-0-78 and HP somaclones (15.50 and 17.34 t ha^{-1} respectively). Paul (2006) reported that the somaclones derived from cultivar Rio-de-Janeiro were high yielding compared to those from cultivar Maran. The higher yield observed in the somaclones derived from polyploid parent Z -0-86 in the present study can be attributed to the original parent Rio-de-Janeiro.

Table 16. Mean rhizome yield of somaclones from each parent

Parents	Fresh rhizome (t ha^{-1})	Dry rhizome (t ha^{-1})
Z-0-86	22.42	4.86
Z-0-78	15.50	3.32
HP	17.34	3.67

5.1.3 Quality attributes

Quality attributes such as driage, volatile oil, oleoresin, starch and crude fibre contents varied significantly in the somaclones. Among forty somaclones, SE HP 8 recorded highest driage (27.13%). Somaclones SE 86 41 (25.40%), C 86 32 (25.06%), SE 78 26 (24.40%), C 86 23 (24.26%), C 86 124 (23.80%), SE HP 9 (23.40%), C 86 8 (22.53 %), C 86 26 (22.47%), SE 86 81(22.26 %) and SE 78 30 (23.33%) also recorded high dry recovery. Generally somaclones derived from Z-0-86 showed higher driage compared to other parents (Table 17).

Recovery of volatile oil varied between 1.2 to 2.4 per cent in the somaclones studied. Nataranjan *et al.* (1972) reported volatile content between 1.25 to 2.81 % in different cultivars of ginger. Among 40 somaclones, SE 86 40 showed highest volatile content, followed by CHP 99 (2.3%) and C 78 284 (2.1%). Lowest volatile oil recovery was observed in somaclones SE 78 26, C 78 13 and SE 86 42 (1.2%). Highest volatile oil yield was observed in the somaclone SE 86 40 (140.56 kg ha^{-1}) and lowest was in the SE HP 74 (24.55 kg ha^{-1}). Compared to mean of somaclones (1.58 %), out of forty somaclones, ten somaclones were significantly superior, thirteen were on par and seventeen were significantly lower in volatile oil content. Shankar (2003) reported higher volatile oil and low fibre content in Z-0-86. But in present study, somaclones derived from HP, showed lower crude fibre content and somaclones derived from Z-0-78, showed higher volatile oil content, however volatile oil yield was higher in somaclones of Z-0-86 (Table 17).

Oleoresin content in a somaclones ranged between 3.28 to 5.94 %. In general, somaclones derived from Z-0-86 showed higher oleoresin content (Table 17). This may be due to the fact that the polyploid Z-0-86 is derived from Rio-de-Janeiro which is a potential cultivar for oleoresin production as observed by Shankar (2003). The results are in agreement with the findings of Paul (2006) who reported higher oleoresin recovery in somaclones of Rio-de-Janeiro (4.38 to 8.93%) than the somaclones of cultivar Maran (4.31 to 8.49%). Among forty somaclones, somaclone C 86 124 (5.94%) showed highest oleoresin, content, followed by SE 86 41(5.44%) and C 86 40 (5.35%). Highest oleoresin yield was observed in the somaclone C 86 23 (336.64 kg ha^{-1}), closely followed by somaclone SE 86 81 (332.71 kg ha^{-1}). Compared to mean of somaclones (4.53%), out of forty somaclones twenty somaclones were significantly superior, six were on par and fourteen had significantly lower oleoresin content.

Crude fibre content ranged between 2 to 3.86 per cent in the somaclones studied. Jogi *et al.* (1972) reported crude fibre content in the range 1.1 to 7.0 per cent

in different cultivars. In general, all the somaclones showed lower fibre content which is considered as a desirable quality attribute for fresh ginger rhizome. Somaclones derived from Z-0-78 showed higher fibre content compared to Z-0-86 and HP (Table 17). Among forty somaclones, C 86 8 showed lowest fibre content (2.00%), closely followed by SE HP 8 (2.10%) and SE HP 74 (2.37%). Higher fibre content was observed in somaclones C 86 139 (3.86%), C 86 124 (3.70%) and C 86 141 (3.62%). Dev (2013) in a previous study with the somaclones of the same parents reported lower mean fibre content in somaclones compared to mean check varieties (3.45%) and parent clones (3.59%). In present study also 14 somaclones showed low mean crude fibre content compared to mean of somaclones (3.07%).

Starch content was observed between 40.59 to 52.56 per cent. Ratnambal *et al.* (1987) reported starch content in the range 40.4 to 59 per cent in different cultivars. In general, somaclones which had high starch content exhibited high driage also. Among forty somaclones, somaclone SE HP 8 showed highest starch content, followed by somaclone C 86 139 (49.00%) and SE 78 26 (48.92%). Lowest starch content was observed in somaclones C 78 381 and SE HP 74 (40.59%). Compared to mean of somaclones (44.23%), out of forty somaclones twelve somaclones were significantly superior, ten somaclones were on par and eighteen somaclones had significantly lower starch content. Lawrence (1984) reported that starch is the most abundant of the constituents of ginger, comprising of 40 to 60 per cent of the weight of the dry rhizome.

As observed in the present study, variation in yield and quality attributes in somaclones of ginger were reported by Bhagyalakshmi *et al.,* (1994), Rao *et al.* (2000), Paul (2006), Shylaja *et al.* (2010), Kurian (2010), Resmi and Shylaja (2012), Dev (2013) and in turmeric by Roopadarshini and Gayatri (2012) and in cardamom by Chandrappa *et al.* (1996). Ravindra *et al.* (2004) reported variability in somaclones of *Pelargonium graveolens* for herb yield and essential oil content. Similar results were also reported in mint by Kukreja *et al.* (1991) and Kukreja *et al.*

(1992) where wide range of somaclonal variation in *Mentha arvensis var. piperascens and Mentha arvensis* (Japanese mint) was found for herb yield and oil yield.

Table 17. Mean quality attributes of somaclones from each parent

Parents	Driage (%)	Volatile oil (%)	Volatile oil yield (kg ha^{-1})	Oleoresin (%)	Oleoresin yield (kg ha^{-1})	Starch (%)	Crude fibre (%)
Z-0-86	21.82	1.53	75.25	4.64	228.60	44.62	3.08
Z-0-78	20.11	1.66	50.75	4.30	113.86	43.63	3.32
HP	21.16	1.61	61.26	4.49	164.54	43.92	2.87

5.1.4. Chemoprofiling of volatile oil and non volatile pungent principles

5.1.4.1 *GC-MS analysis of ginger volatile oil*

The volatile oil present in the rhizomes is responsible for the pleasant aroma of ginger and the end products. Thus the essential oil composition determines the quality of the ginger and price of the produce. The essential oil profile of the 11 ginger somaclones revealed presence of total 44 compounds belonging to different chemical groups mainly sesquiterpene hydrocarbons, monoterpene hydrocarbons, monoterpene alcohols, sesquiterpene alcohols etc. Among different groups sesquiterpene hydrocarbons, were major (Tonnessen and Karlsen, 1983) and in sesquiterpene hydrocarbons, zingiberene was the major compound (23.28%). The specific aroma of ginger is predominantly related to zingiberene, as reported by Sultan *et al.* (2005). After zingiberene, alpha-faresene (14.73%), beta-sesquiphellandrene (12.98%) and ar-curcumene (8.4%) were the major ones (Fig 3). Vernin and Parkanyi (1994) reported that zingiberene, alpha-farnesene, beta-sesquiphellandrene and ar-curcumene were major components in Indian ginger essential oil and their concentration ranged from 17.50 to 29.50%, 9.8 to 16%, 7.3 to 9.0% and 8.0 to 17.0% respectively. However in somaclones C 86 124 and C 86 32 zingiberene content was less (15.47 and 15.50% respectively) compared to other

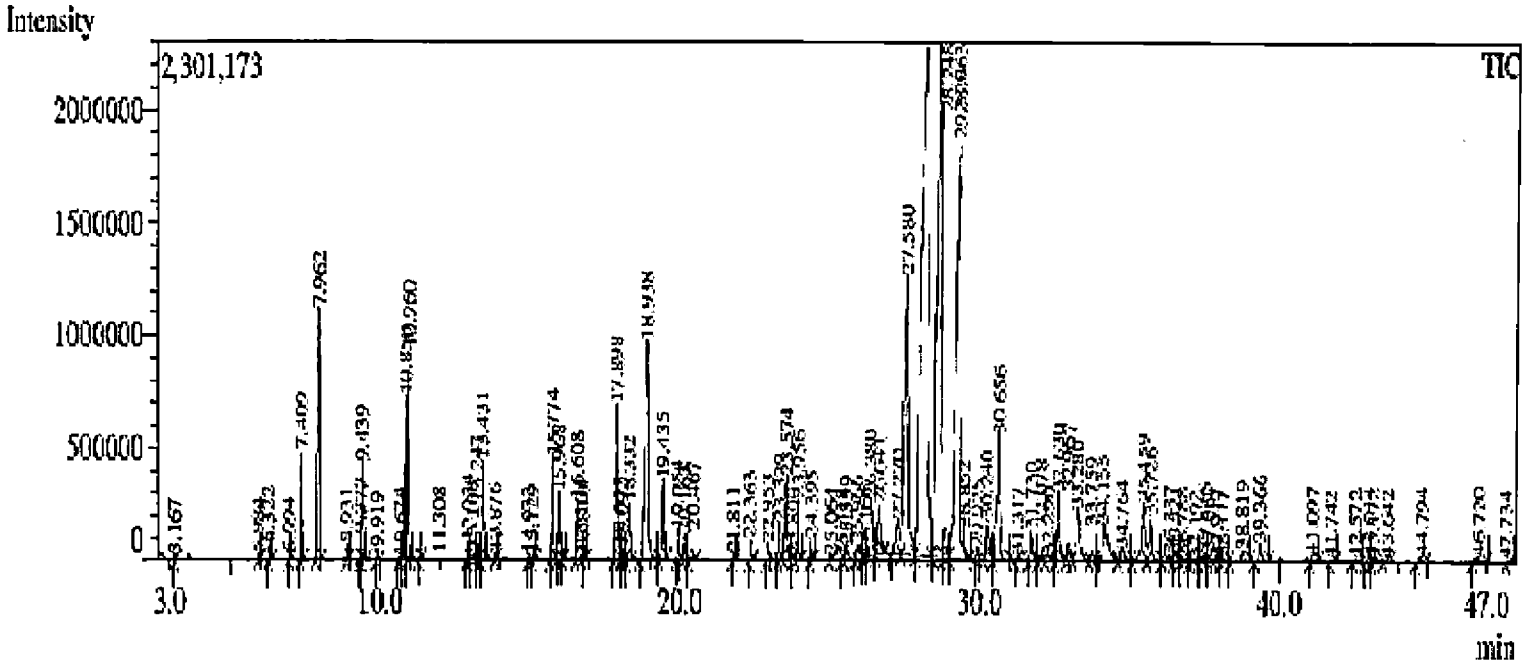


Fig: 1 GC-MS profile of volatile oil from ginger somaclone SE 86 81

somaclones where as ar-curcumene content was high (11.32 and 11.05% respectively). Sankarikutty *et al.* (1982) reported that ar-curcumene is a secondary product formed from zingiberene and beta-phellandrene, in the dried samples of ginger. With respect to oxygenated monoterpenes, borneol and geraniol are present at lower concentrations. The monoterpene hydrocarbons, camphene and beta-phellandrene and sesquiterpene alcohols, nerolidol and alpha-eudesmol are also present in the extracted oil. As indicated earlier, the main constituents in the oil of the somaclones also belong to the sesquiterpene hydrocarbons. Sultan *et al.* (2005) carried out works concerning the composition of Thai ginger essential oils by GC equipped with Flame Ionization Detector and found that zingiberene (30.81%) constituted the major fraction of the oil followed by citral (5.4%), myrcene (4.6%), 1,8- cineol (3.9%), N-pinene (3.6%), O- phellandrene (2.8%), Y-terpinene (2.5%) and O-pinene (0.74%). Analysis of Chinese ginger essential oils indicated similar qualitative and quantitative chemical composition. Gurdip *et al.* (2008) analyzed the chemical composition of ginger essential oil by GC– MS and five major components were identified; geranial (25.9%); a-zingiberene (9.5%), (E,E)-a-farnesene (7.6%), neral (7.6%) and ar-curcumene (6.6%). In the present study, wide variability was observed for four major aroma compounds among the somaclones, zingiberene 15.47 (C 86 124) to 29.64 % (CHP 99), beta-sesquiphellandrene 11.56 ((C 86 124) to 14.69% (CHP 99), alpha-farnesene 3.33% (C 86 124) to 17.86% (C 86 139) and ar-curcumene 3.98% (SE 86 83) to 11.32% (C 86 124). These findings are in agreement with the report of Kukreja *et al* (1992) that wide range of somaclonal variation existed in *Mentha arvensis var. piperascens* for menthol (65.2-94.8%), menthone (1.4-20.9%), isomenthone (1.0-5.2%) and menthylacetate (0.8-8.5%). Ravindra *et al.* (2004) also reported variability in somaclones of *Pelargonium graveolens* for oil components, such as linalool and trans-rose oxide. Variability in the components of oil depends on several factors, mainly extraction methods, type of solvents, geographical area,

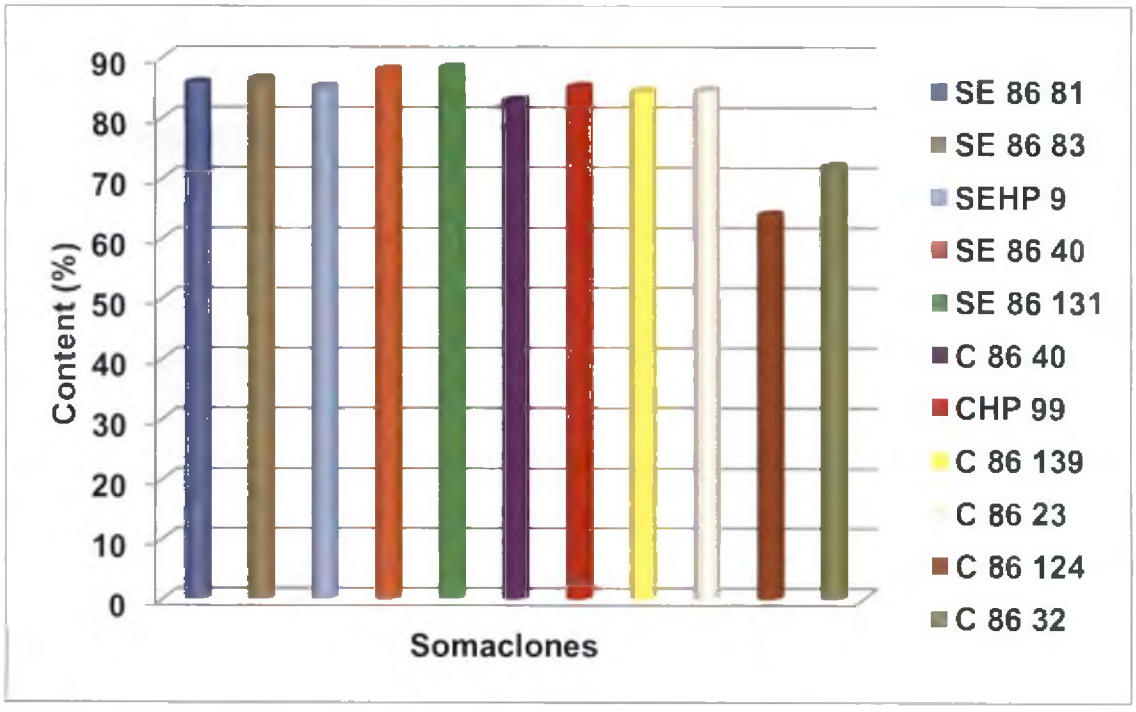


Fig 2. Percentage of volatile oil constituents in ginger somaclones

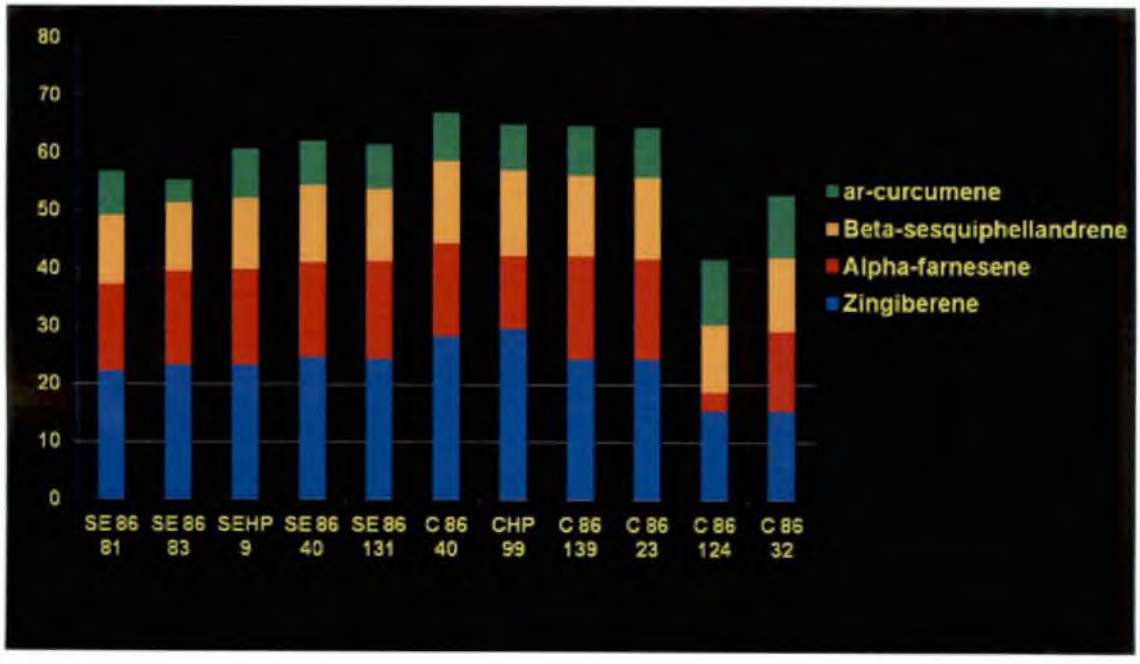


Fig 3. Content of important sesquiterpene hydrocarbons in volatile oil of ginger somaclones

varietal property and type of rhizome (fresh or dried rhizome) as reported by He *et al.* (2001) and Singh *et al.* (2008) in ginger.

Few compounds were detected only in some somaclones and they are unique to the somaclones. Beta-citronellyl acetate, aromadendren and beta – biasbolene were unique compounds in somaclone SE 86 81 and compounds germacrene d and beta-phellandrene were unique to the somaclone SE 86 83. The compounds limonene, linalool gamma-cadinene were unique compounds in somaclones C 86 23, SE HP 9 and C 86 32 respectively.

5.1.4.2 *HPLC analysis of non volatile pungent principles*

Gingerols and shogaols are pungency stimulating non-volatile compounds present in ginger. Apart from pungent activity gingerols and shogaols are also known for their pharmaceutical properties. Among gingerols, 6-gingerol is the most biological active compound known for its antioxidant, antipyretic, antiseratogenic, antiulcer and cardio depressant property. With respect to shogaols, 6-shogoal is most important and it is known for its antiallergic, antioxidant, antiprostaglandin and CNS-depressant property. The variability of gingerols and its related compounds in twelve ginger somaclones was assessed by HPLC techniques. The study showed that in all somaclones gingerols were major compounds compared to shogaol (Fig 4) Jiang *et al.* (2006) also reported similar results. Somaclone C 86 124 showed highest gingerols content (2.68%) compared to others and somaclone C 86 139 showed lower gingerol content (1.40%). Ratio of gingerol to shogaol decides quality of the ginger, and pungency of ginger gradually decreases when the amount of gingerol decreases and shoagaol increases (Zachariah *et al.,* 1993). In the present study, gingerols and shogaols ratio was observed between 3.76:1 to 7.92:1, with the highest ratio in somaclones SE 86 42 (7.92:1) followed by SE 78 26 (7.87:1) and lowest ratio in somaclone C 86 40 (3.76:1), followed by SE HP 9 (4.03:1). Connel (1970) reported that storage of oleoresin can result in chemical conversion of gingerols to non

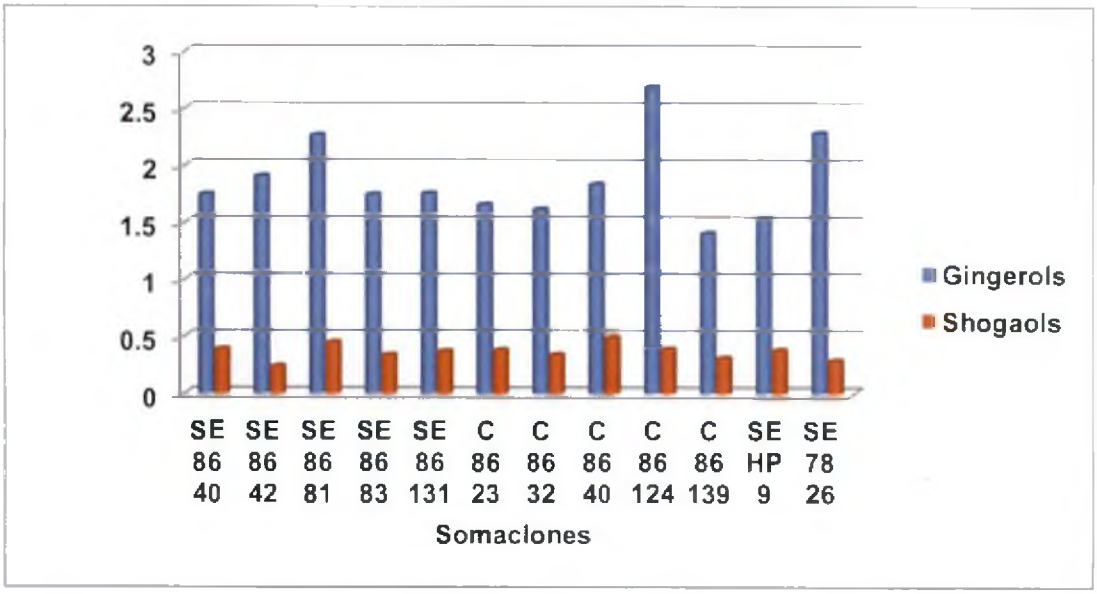


Fig 4. Content of pungent principles in ginger somaclones

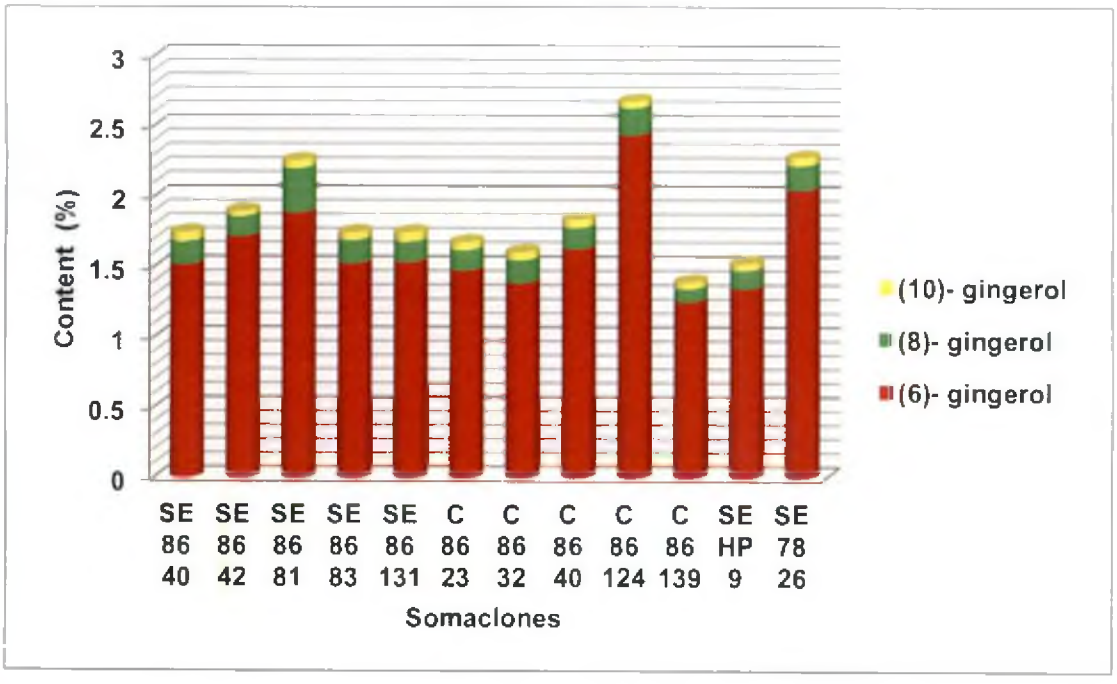


Fig 5. Concentration of gingerols in ginger somaclones

pungent shogaols and this conversion is undesirable with respect to quality because of loss of pungency, development of off flavour and accumulation of non pungent residue. Present study using HPLC observed meager levels of shogaols in all the somaclones of ginger; supporting the finding of Jolad *et al.* (2005) who reported low shogaols compounds in ginger oleoresin. Among twelve somaclones, C 86 40 showed higher shogaols content of 0.49 % and lower shoagaols content was in the somaclone SE 86 42 (0.24%).

Among the gingerols, 6-gingerol was the most abundant pungent compound (Fig 5) and highest 6-gingerol obtained was 2.44 % in the somaclone C 86 124. Somaclones showing 6-gingerol content above 1.5 % were SE 86 40, SE 86 42, SE 86 81, SE 86 83 and SE 86 131. Chen *et al.* (1986) and Bartley (1995) also reported similar results. Kizhakkayil and Sasikumar (2012) characterized 46 ginger accessions based on non volatile compounds and reported 6-gingerol content ranging from 3.11% (Angamali) to 0.36% (Oman) and shogaols content from 0.23 % (Palai) to 1 % (Oman). Chen *et al.* (1986) reported total gingerol content (6-, 8- and 10-gingerol) of 0.65–0.88% (w/w) in green ginger and 1.10–1.56% (w/w) in dry ginger.

5.1.5 Changes in quality attributes with crop maturity

The quality changes with respect to volatile oil, oleoresin, starch and crude fibre contents at 180 days and 240 days of planting was assessed. Significant variation in quality attributes was observed with changes in maturity of rhizomes.

5.1.5.1 *Volatile oil*

Volatile oil content decreased with increase in crop duration. Irrespective of the somaclones, volatile oil content was highest at 180 days compared to full maturity (Fig 6). Among seven somaclones, somaclone (SE 86 40) had maximum volatile oil content at 180 and 240 days after planting (3.45% and 2.40% respectively). Accumulation of starch and *in vitro* loss of volatiles decrease the essential oil content during ontogenesis of rhizomes (AOAC, 1965). The presence of essential oil in the

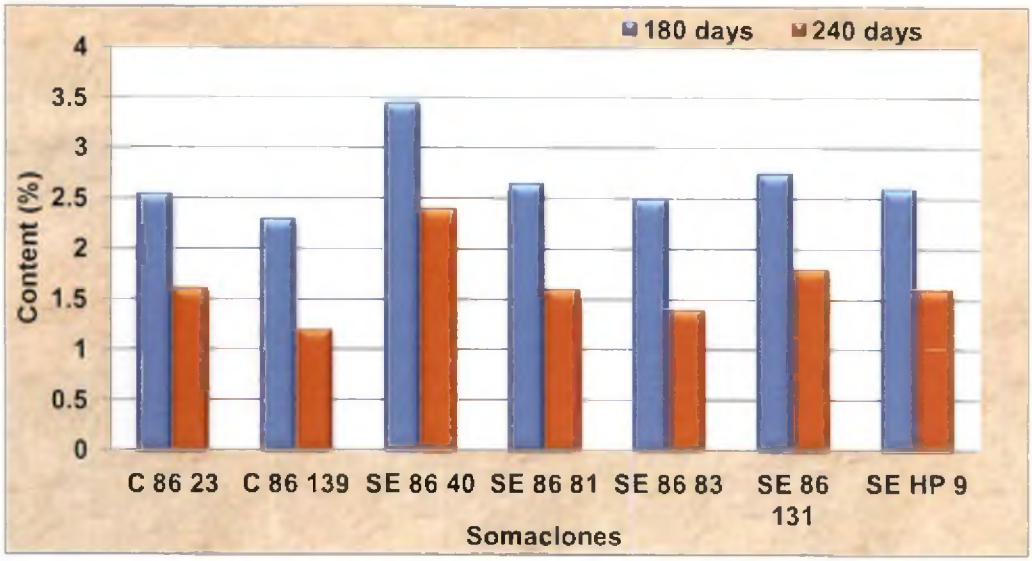


Fig 6. Changes in volatile oil content with crop maturity of ginger somaclones

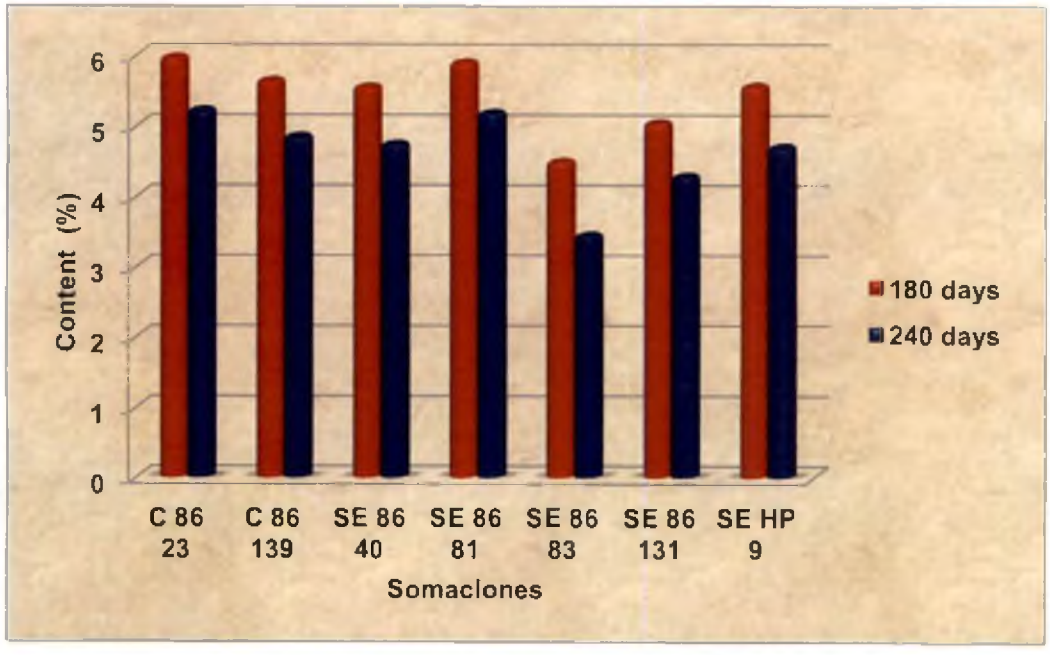


Fig 7. Changes in oleoresin content with crop maturity of ginger somaclones

outer skin established by histochemical examination of ginger peel confirmed decreasing levels of essential oil during rhizome development (Mangalakumari *et al.*, 1984). Sanal *et al.* (2010) and Jogi *et al.* (1972) also reported similar trend for essential oil accumulation in ginger.

5.1.5.2 *Oleoresin*

Oleoresin content showed a decreasing trend with increase in maturity (Fig 7). Oleoresin content in all somaclones was highest at 180 days compared to 240 days of planting. Somaclone C 86 23 showed highest oleoresin content at 180 days and also at 240 days of planting (5.97 and 5.23 % respectively). Lowest oleoresin content at both harvesting stages (180 and 240 days of planting) was in somaclone SE 86 83 (4.50 and 3.45 % respectively). Mathai (1972) reported that on dry weight basis, accumulation of oleoresin content decreased with maturity. Nataranjan *et al.* (1972) reported that though the percentage of essential oil and oleoresin decreased with maturity, the final yield per hectare of these quality components was highest at maturity.

5.1.5.3 *Starch*

With the advancement of maturity, the starch content followed an increasing trend (Fig 8). In all the somaclones, starch content was higher at 240 days compared to 180 days. It was almost double at full maturity than at 180 days. Somaclone C 86 139 showed highest starch content (49.00%) at 240 days of harvesting and minimum starch content (41.71%) was observed in somaclone SE 86 131 at 240 days of planting. As observed in the present study, Sanal *et al.* (2010) reported that dry recovery, starch and crude fibre contents will increase towards maturity. Ratnambal *et al.* (1987) also reported similar results.

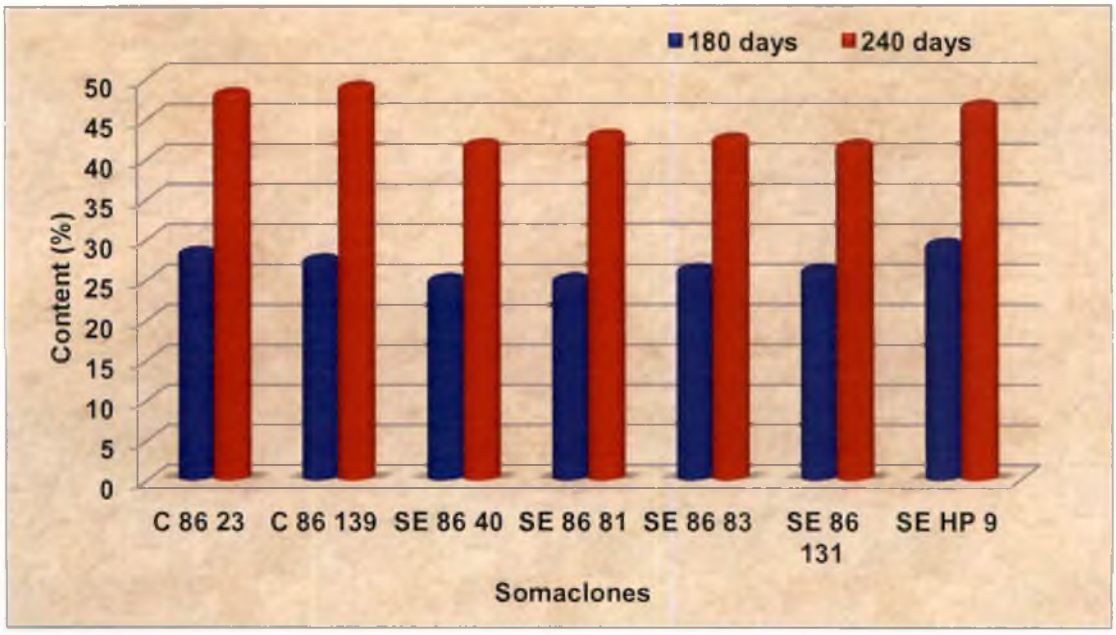


Fig 8. Changes in starch content with crop maturity of ginger somaclones

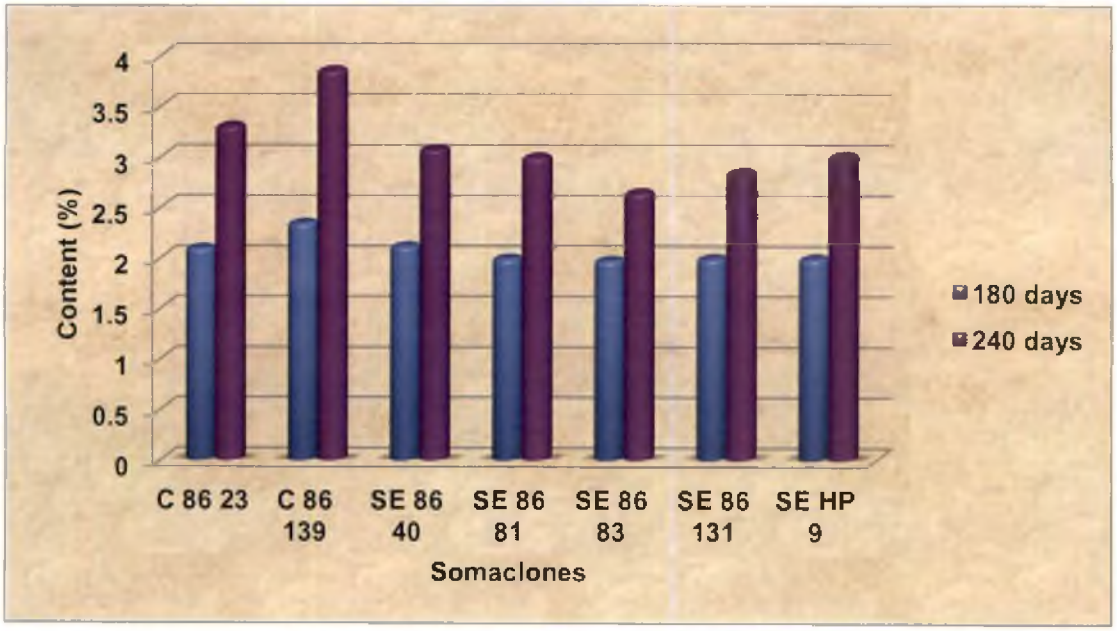


Fig 9. Changes in crude fibre content with crop maturity of ginger somaclones

5.1.5.4 *Crude fibre*

On an average, the crude fiber content of seven somaclones at 180 days was 2.08 % and increased to 3.11% at 240 days (Fig 9). Somaclones C 86 139 and C 86 23 showed higher fibre content at 180 and 240 days after planting. It was observed that SE 86 83 had lower fibre content at both stages. Sanal *et al.* (2010) reported that though increase in fiber content was noticed up to the last stage of harvesting, maximum rise in the fiber content was observed between 150 to 180 days of planting. Jogi *et al.* (1972) and Ratnambal *et al.* (1987) also reported similar trend for crude fibre.

5.2 SCREENING SOMACLONES FOR VALUE ADDITION

The selected somaclones were screened for suitability for preparing ginger candy and paste. Seven somaclones each were selected for preparation of ginger candy (SE 86 40, SE 86 81 SE 86 83, SE 86 131, C 86 23, C 86 139 and SE HP 9) and ginger paste (SE 86 40, SE 86 81 SE 86 83, SE 86 131, C 86 23, C 86 124 and SE HP 9). The preparation of ginger candy was done at Nadukkara Agro Processing Ltd., Avoli, Ernakulam and ginger paste at M/s Manjilas Food Products, Thrissur following the procedure adopted by them.

5.3 STABILITY OF QUALITY PARAMETERS OF GINGER CANDY AND PASTE DURING STORAGE

The prepared ginger candy and paste were packed in plastic bottles and three layered aluminum pouches respectively and stored under ambient conditions for three months. The physico-chemical characteristics and microbial load in the products were recorded immediately after preparation of products and after three months of storage.

5.3.1 Physical characteristics

5.3.1.1 *Moisture*

Initial moisture content of ginger candy and paste varied between 9.88 to 11.11% and 65 to 67.4% respectively. In ginger candy, somaclone SE HP 9 showed highest moisture content 11.10 (%). Minimum moisture content was observed in somaclones C 86 139, SE 86 83, SE 86 131 and control (9.89%). Sivakumar (2013) reported similar results where moisture content of amla candy was 13.14 %. However 37% moisture content was reported by Anis (2012) in ginger candy, which is a much higher value compared to the results of the present study. In ginger paste, highest moisture content was observed in somaclone SE HP 9 (67.40%) and somaclone C 86 124 showed minimum moisture content (65.10%). With respect to ginger paste, Priya *et al.* (2010) reported similar results where moisture content of ginger-garlic paste was (75.1%).

Moisture content of ginger candy and paste showed a decreasing trend upon storage, irrespective of the somaclones (Fig 12 and 13). Somaclone SE 86 81 showed maximum moisture loss of 8.22 (%) in candy after 3 months of storage and minimum of 4.60 per cent was observed in somaclone SE HP 9. In paste category, somaclone SE 86 131 and C 86 124 showed minimum and maximum moisture loss of 0.44 and 0.56 % respectively. Sagar *et al.* (2000) and Sivakumar (2013) also reported moisture loss in mango powder and amla candy respectively, upon storage. Decrease of moisture in both ginger paste and candy may be due to changes in weather conditions during storage and evaporation loss of moisture from the product. Reduction in moisture content may impart increase in TSS, retention in quality and extended shelf life.

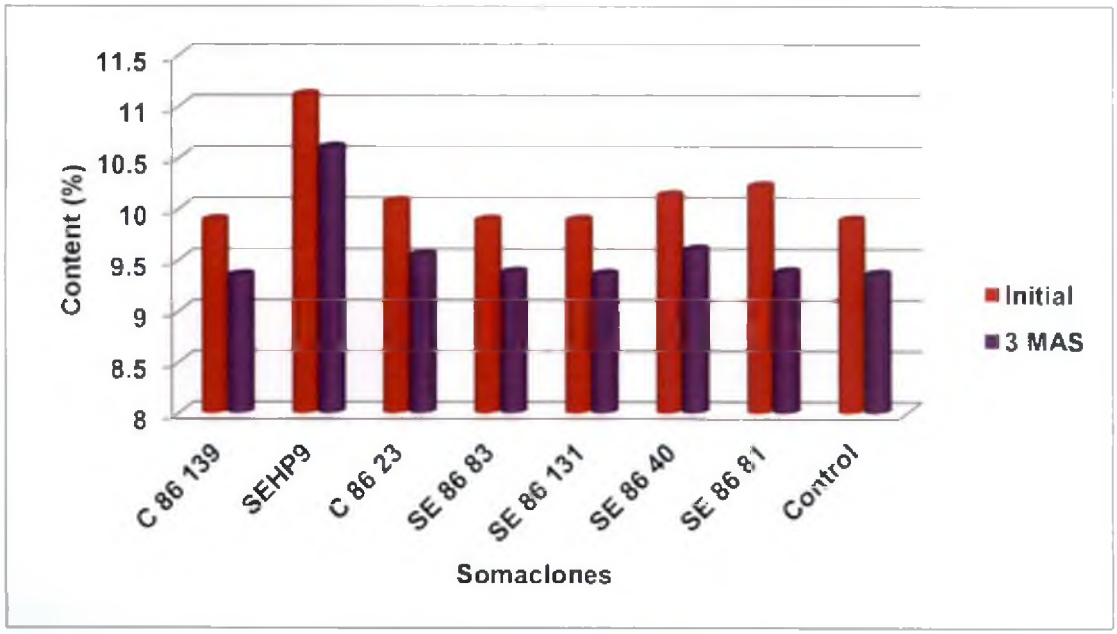


Fig 12. Moisture content of ginger candy from different somaclones and control at initial and three months of storage

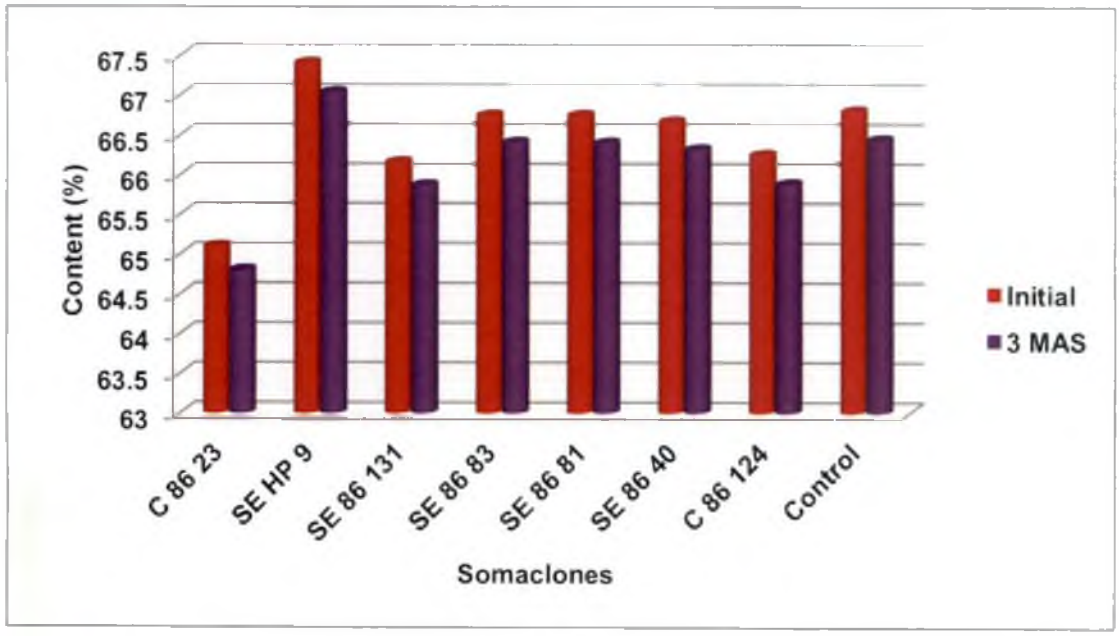


Fig 13. Moisture content of ginger paste from different somaclones and control at initial and three months of storage

5.3.1.2 *Colour*

In ginger candy, initially all the somaclones and control showed same colour of light yellow (18b). With respect to ginger paste, initially there was no significant difference in colour between somaclones except SE 86 83, which had pale creamy yellow colour (13B) and others along with control had pale yellow colour (12D).

In ginger candy compared to initial colour, there was slight colour change during storage, in the all candy somaclones and control. Ginger candy from somaclones C 86 139, SE HP 9 and C 86 23 showed light yellow colour (20B) after 3 months storage whereas candy from somaclones SE 86 83, SE 86 131, SE 86 40, SE 86 81 and control showed light yellow colour (18A). No significant difference was observed in ginger paste somaclones even after 3 months of storage. Slight increase in non enzymatic browning and polyphenol oxidase activity during storage might be responsible for slight colour change in both ginger candy and paste upon storage. The ginger candy and paste from somaclone SE 86 131 recorded lowest non enzymatic browning and polyphenol oxidase activity even after storage indicating the cultivar influence on product stability.

5.3.2 Biochemical parameters

5.3.2.1 *TSS*

Initial TSS content of ginger candy and paste were reported between 68.5 to 69 ^{0}B and 24.27 to 25.75 ^{0}B respectively. Mishra *et al.* (2013) reported TSS content of 73.8 ^{0}B in bael candy. Sivakumar (2013) also reported similar results in amla candy. Cultivar differences in the TSS content of ginger candy was less when compared to ginger paste.

TSS content in both ginger candy and paste showed increasing trend during storage (Fig 14 and 15). Somaclone SE 86 83 showed highest TSS content (69.44 ^{0}B) in ginger candy after 3 months of storage. Minimum TSS content was observed in

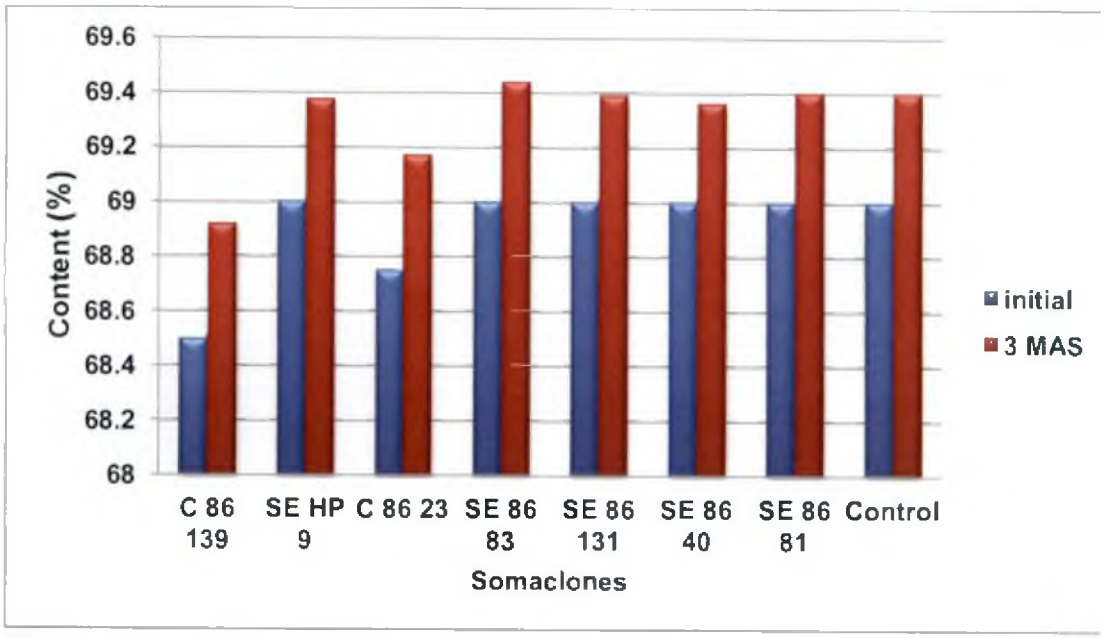


Fig 14. TSS content of ginger candy from different somaclones and control at initial and three months of storage

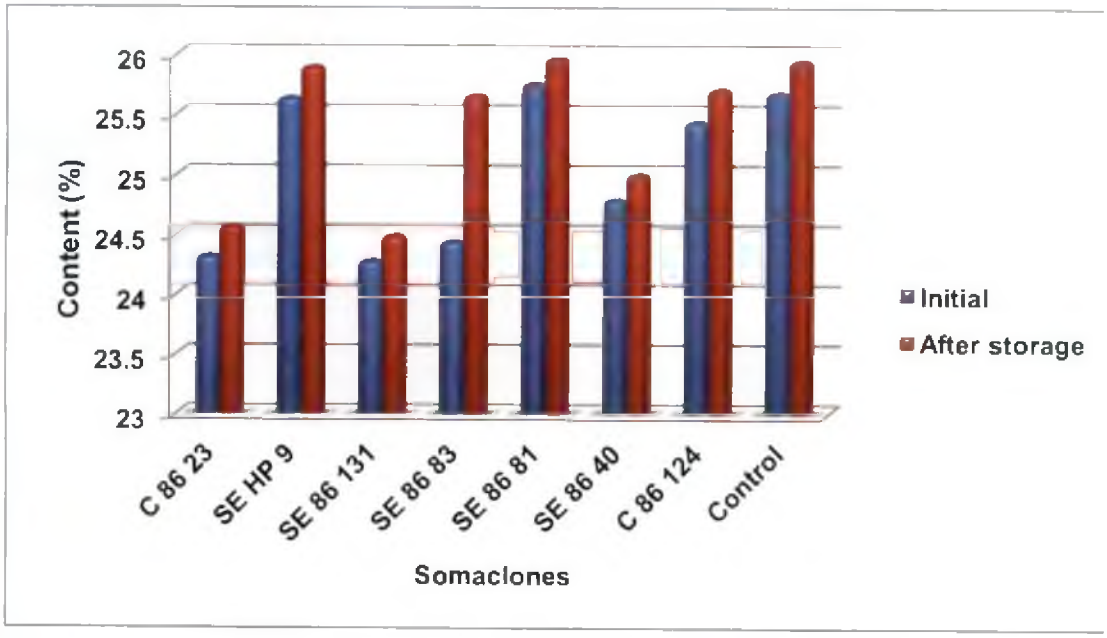


Fig 15. TSS content of ginger paste from different somaclones and control at initial and three months of storage

somaclone C 86 139 (68.92 ^{0}B). In paste, control recorded maximum TSS (25.94^{0}B) and somaclone SE 86 131 showed minimum TSS (24.56 ^{0}B) after storage. Increase in TSS is due to conversion of polysaccharides to sugars and it can be attributed to reduction in moisture content of the product with advanced storage period. Increase in TSS was also observed in bael candy by Mishra *et al.* (2013) and Sivakumar (2013) in amla candy, where TSS content increased from 57.10 to 58.20 °bx after 9 months of storage. Jasim (2004) reported that TSS, sodium chloride, titratable acidity and pH of ginger paste did not change significantly ($P > 0.05$) during storage.

5.3.2.2 *pH*

Initial pH values of ginger candy and paste were between 3.51 to 3.60 and 3.61 to 3.75 respectively. In ginger candy, maximum pH (3.60) was observed in somaclone SE 86 131 and minimum pH (3.51) was observed in somaclone SE 86 83 and somaclone SE 86 40. In ginger paste, somaclone SE 86 81 showed maximum pH (3.75) and somaclone SE 86 131 showed minimum pH (3.62). Sivakumar (2013) reported pH content of 3.44 in amla candy. Jasim (2004) reported a pH of 4.05 in ginger paste.

pH content of ginger candy and paste showed decreasing trend during storage (Fig 16 and 17). Somaclones did not have any significant effect on pH of ginger paste during storage. Algadi *et al.* (2014) reported that variety had no effect on the pH of garlic paste as the two varieties (Dongola and Berber) had similar pH values after storage.

In ginger candy, after 3 months of storage highest pH (3.57) was in somaclone SE 86 131 and lowest pH (3.48) was in somaclone SE 86 83. In ginger paste, somaclone SE 86 81 showed highest pH (3.73) and somaclone SE 86 131 showed lowest pH (3.61). Sivakumar (2013) reported a very slight change of pH (3.44 to 3.38) in amla candy during storage. Jasim (2004) reported similar results in ginger

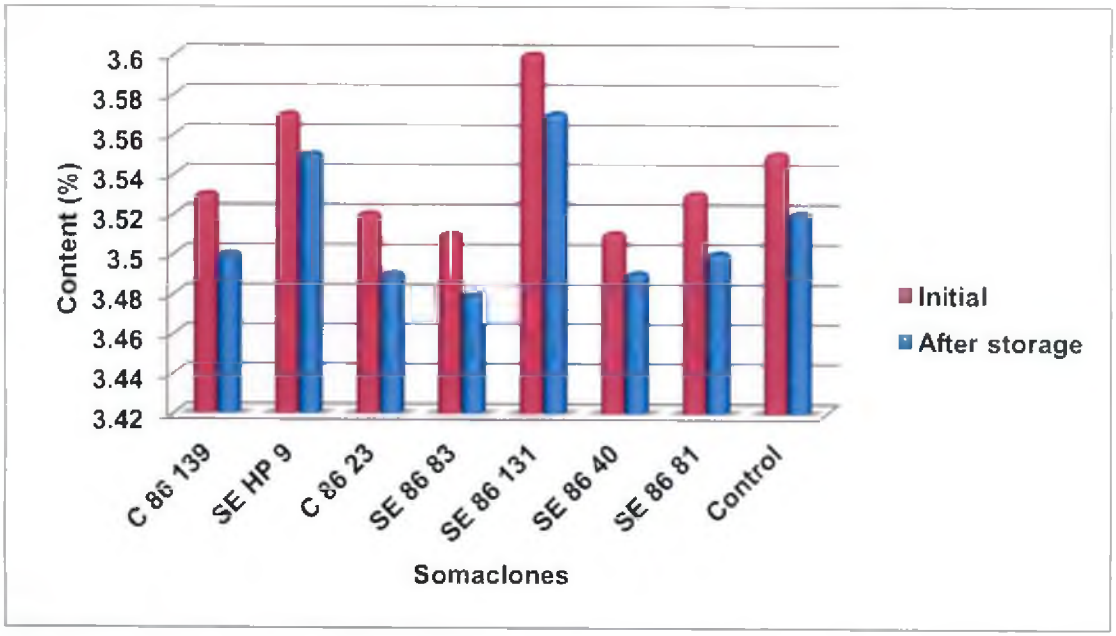


Fig 16. PH of ginger candy from different somaclones and control at initial and three months of storage

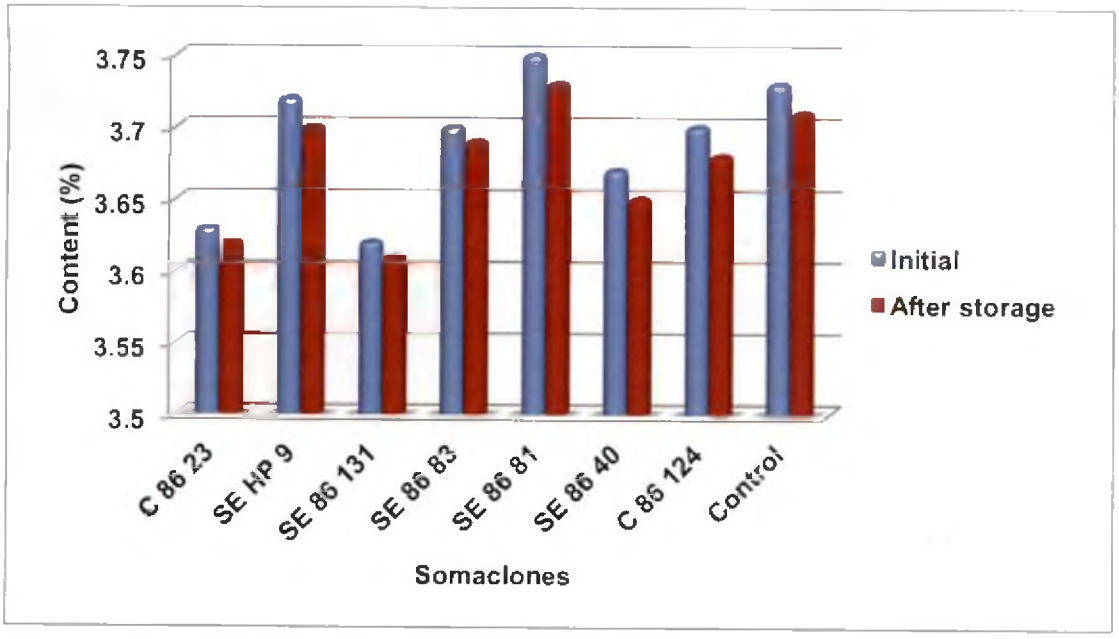


Fig 17. PH of ginger paste from different somaclones and control at initial and three months of storage

paste too. Decrease of pH upon storage can influence quality of the products through suppressing growth of microbes and PPO activity.

5.3.2.3 *Titratable acidity*

Initially, the titratable acidity in ginger candy and paste recorded among the somaclones ranged between 0.16 to 0.19 % and 3.30 to 3.75% respectively. Mishra *et al.* (2013) reported titratable acidity of 0.38 per cent in bael candy. In ginger paste, Jasim (2004) reported titratable acidity of 0.32% and which is notably a lower value compared to the results of the present study. This might be due to the variation in the quantity of ingredients such as acetic acid, added to the product.

Both ginger candy and paste showed an increasing trend in titratable acidity during storage, irrespective of somaclones (Fig 18 and 19). In ginger candy, somaclones C 86 139 and SE 86 81 showed highest acidity (0.26 %), after three months. However, somaclone SE HP 9 and SE 86 131 showed lowest acidity content (0.19%). In ginger paste, somaclone SE 86 131 and somaclone C 86 23 recorded highest acidity of 3.95 and 3.82 % respectively. Degradation of pectic substances into soluble solids might have contributed towards an increase in acidity and also due to interconversion of sugars and other chemical reactions (Clydesdale, 1972). Ramalingam *et al.* (2010) found slight increase in titratable acidity of tropical fruit bars during storage. Mishra *et al.* (2013) observed that per cent TSS, acidity and browning of bael candy increased while ascorbic acid decreased during storage. Lukes (1986), Rejano *et al.* (1997), Ahmed *et al.* (2001) and Jasim (2004) have reported that TSS, sodium chloride, titratable acidity and pH of ginger paste did not change significantly ($P > 0.05$) during storage.

5.3.2.4 *Non enzymatic browning*

Initially non enzymatic browning in ginger candy and paste were observed between 0.090 to 0.125% and 0.129 to 0.160 % respectively. In ginger candy, highest

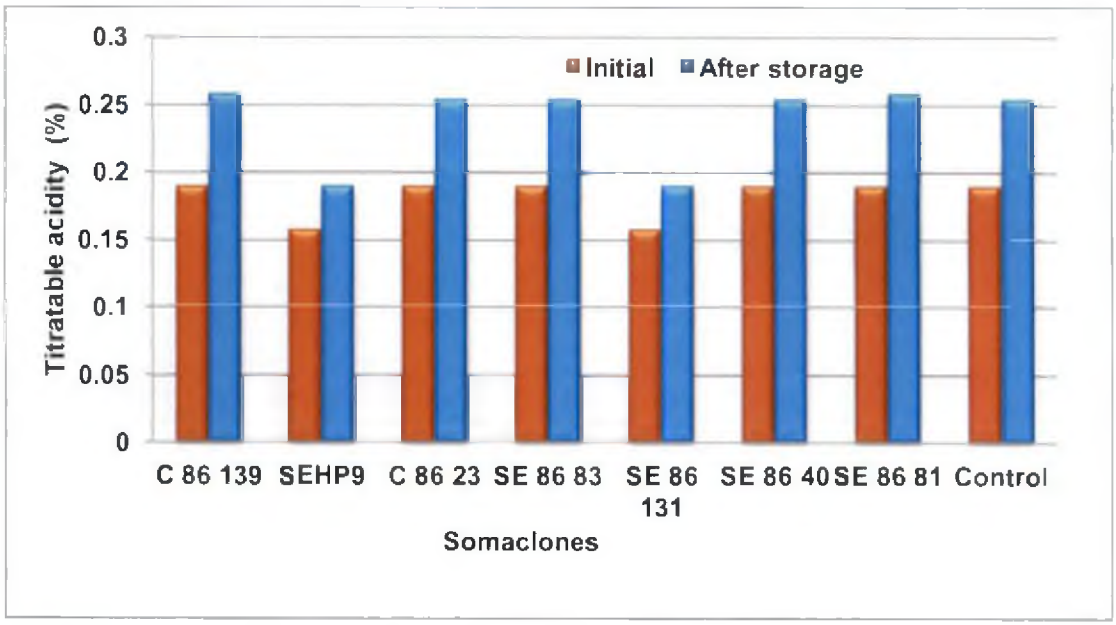


Fig 18. Titratable acidity of ginger candy from different somaclones and control at initial and three months of storage

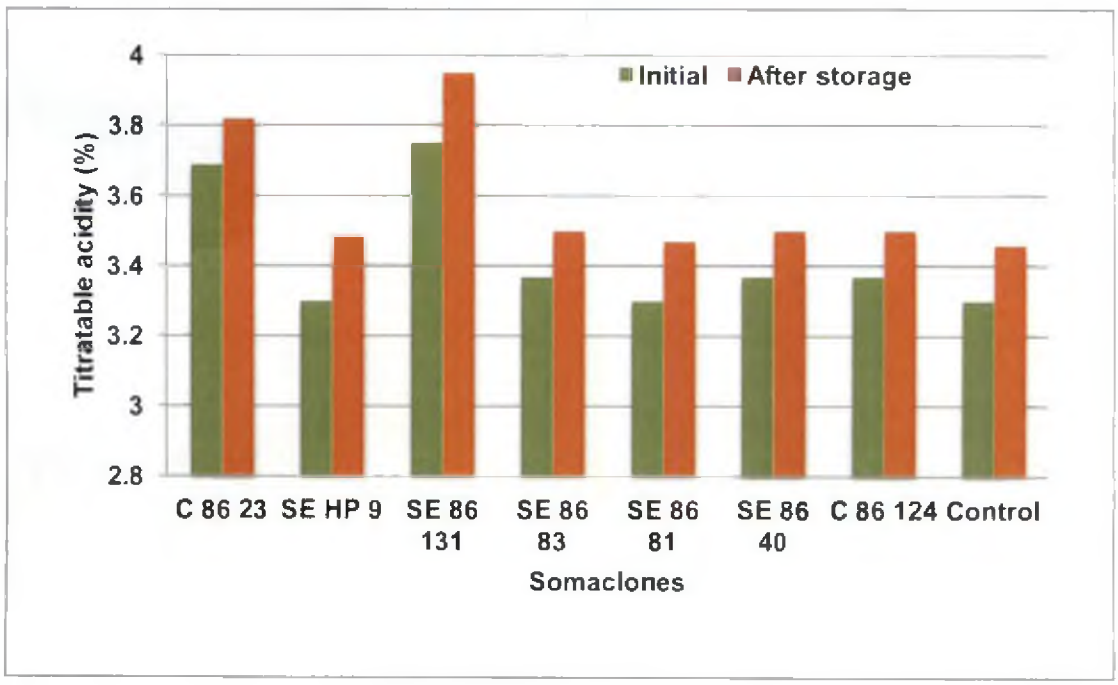


Fig 19. Titratable acidity of ginger paste from different somaclones and control at initial and three months of storage

non enzymatic browning was observed in somaclone C 86 139 (0.125%) and lowest non enzymatic browning was in somaclone SE 86 131 (0.09). In paste, maximum non enzymatic browning (0.160%) was observed in the somaclone C 86 23, while the somaclone SE 86 131 had the minimum non enzymatic browning of (0.129%). Mishra *et al.* (2013) reported 0.02 (absorbance) of non enzymatic browning in bael candy.

Non enzymatic browning increased in both ginger candy and paste during storage (Fig 20 and 21). Candy from somaclone C 86 139 showed maximum (0.150 absorbance) non enzymatic browning after 3 months of storage and somaclone SE 86 131 had minimum (0.113 absorbance) non enzymatic browning. In case of ginger paste, somaclone SE 86 83 had maximum (0.181 absorbance) non enzymatic browning and somaclone SE 86 131 had minimum non enzymatic browning (0.145 absorbance).

The browning of candy was mainly due to non-enzymatic reactions such as organic acid with sugar and /or oxidation of phenols, which lead to formation of brown pigments (Mishra *et al,* 2013). Potter (1989) reported that there was increase in non enzymatic browning of mango leather when stored for 70 days. Non enzymatic browning plays an important role in quality of the products. Quality of the processed product decreases with increase of non enzymatic browning because of formation of brown pigments. In present study non enzymatic browning was low in both ginger candy and paste even after storage for three months indicating the storage stability of products.

5.3.2.5 *Polyphenol oxidase (PPO) activity*

Polyphenol oxidase activity was absent totally soon after preparation in ginger candy and paste, irrespective of somaclones (Fig 22 and 23). But PPO activity was observed after three months of storage and the candy and paste from different

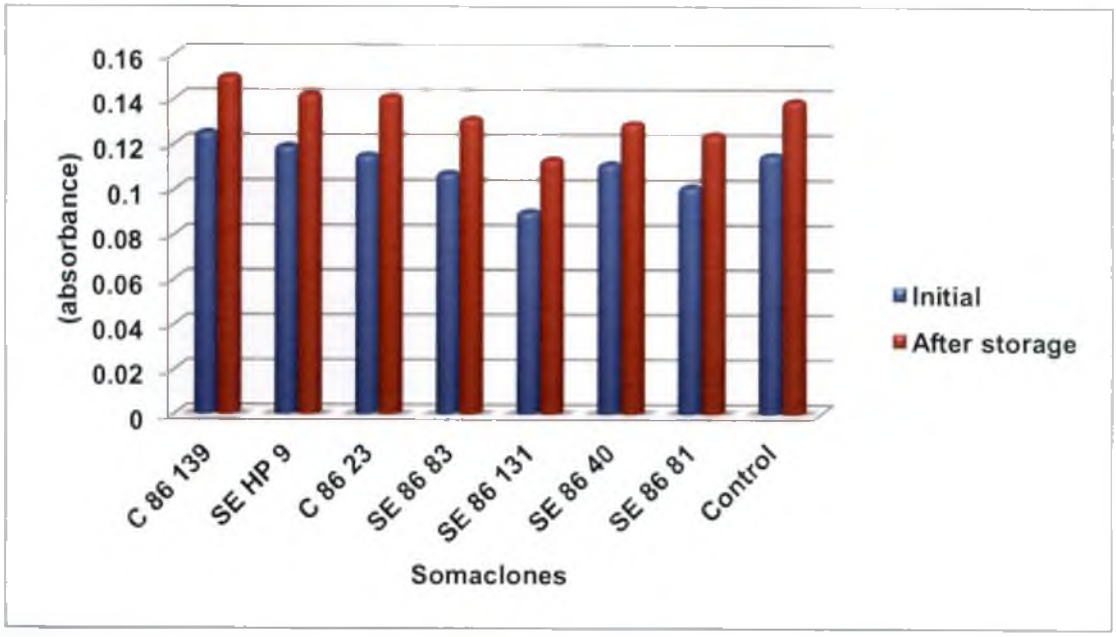


Fig 20. Non enzymatic browning of ginger candy from different somaclones and control at initial and three months of storage

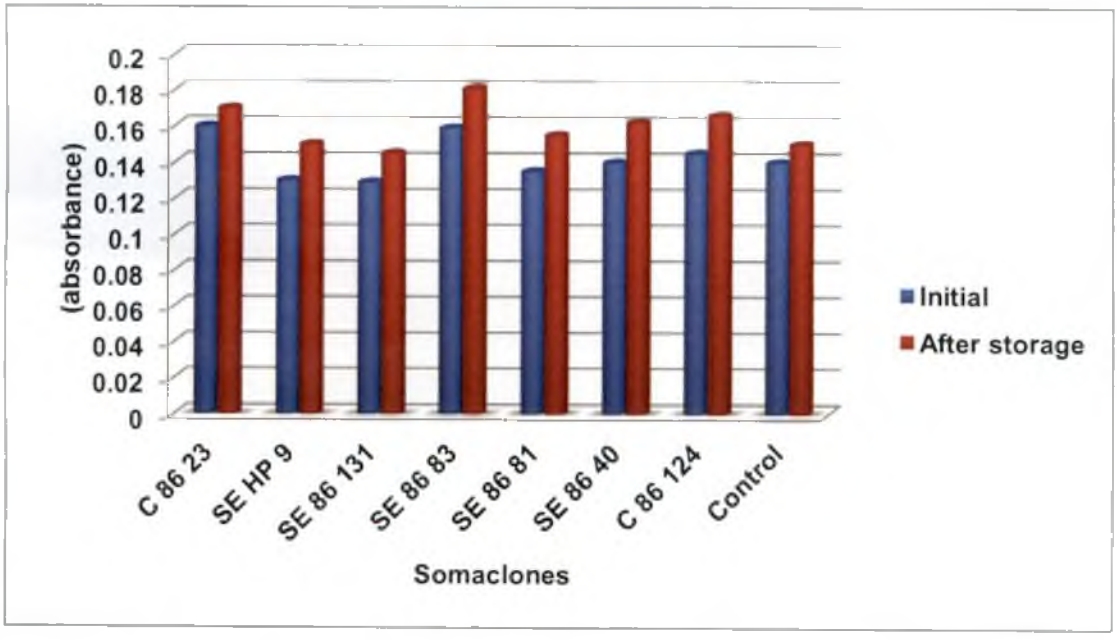


Fig 21. Non enzymatic browning of ginger paste from different somaclones and control at initial and three months of storage

somaclones differed significantly. In ginger candy highest PPO activity was observed in somaclone C 86 23 (0.25 units/litre) and lowest was in somaclone SE 86 131 (0.08 units/litre). In ginger paste, somaclone C 86 23 showed higher (0.98 units/litre) polyphenol oxidase activity and somaclone SE 86 131 showed lower PPO activity (0.25 units/litre).

Initially no PPO activity was detected in any of the candy and paste samples because of high temperature and chemical preservatives used during preparation. Even after storage for three months the PPO activity was very meager. Jen and Kahler (1974) reported that temperature above 80^0C will inactivate PPO activity. Apart from temperature, pH of the products is also as important factor for effective control of PPO activity. Both ginger candy and paste had pH < 4.0, which is unsuitable for PPO activity. The results are in agreement with the findings of Chen and Pei (2014) where they reported that the optimum pH of the enzyme was 7.5 and that enzyme activity decreased rapidly at pH below or above the optimum (7.5) while investigating the effect of pH on the activity of PPO in cassava leaf.

5.3.3 Microbial load in ginger candy and paste

Both candy and paste showed a slight increase in microbial load during the storage period. Initially no microbial load was observed in candy and paste samples from any of the somaclones. In ginger candy microbial load increased to 2-5 x 10^5 bacterial counts, 1-2 x 10^3 mould counts and 1-2x10^3 yeast count at the end of storage period. In ginger paste, microbial load increased to 1-2 x10^5 bacterial counts, 1 x 10^3 mould counts and 1x 10^3 yeast counts. However, microbial population has been observed in ginger candy and paste it was in an acceptable limit. The meager increase in the microbial count indicated the pre processing, processing and storage might have been in a hygienic condition and so the environment was not found suitable for rapid microbial growth. Apart from this, addition of preservatives and high processing temperature also might have controlled the microbial growth. Garriga *et*

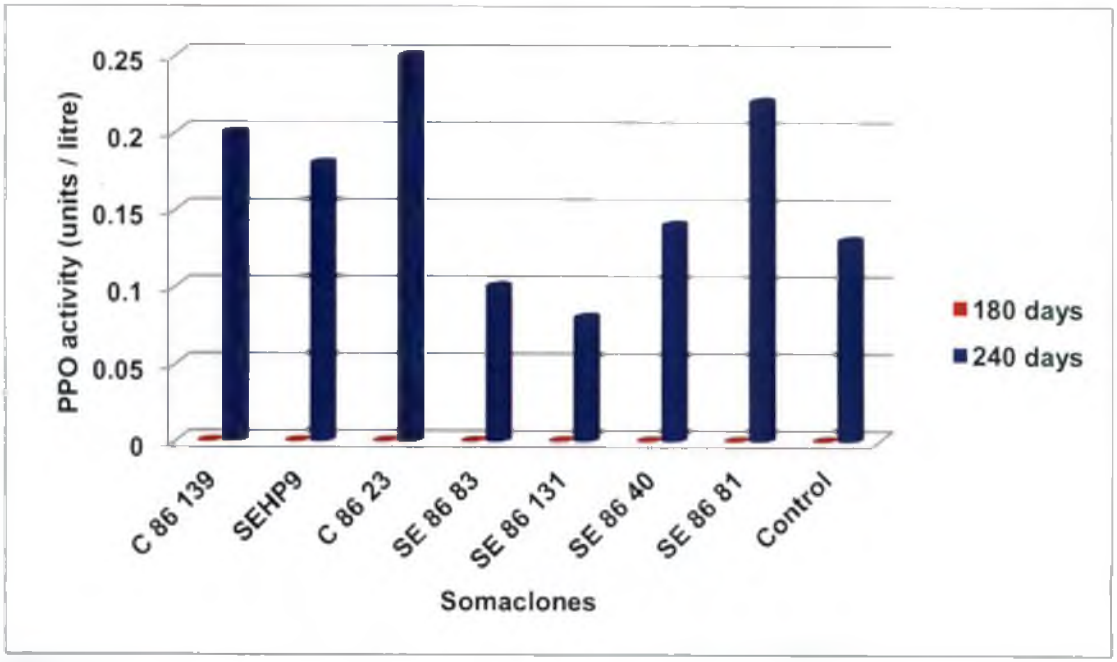


Fig 22. Polyphenol oxidase activity of ginger candy from different somaclones and control at initial and three months of storage

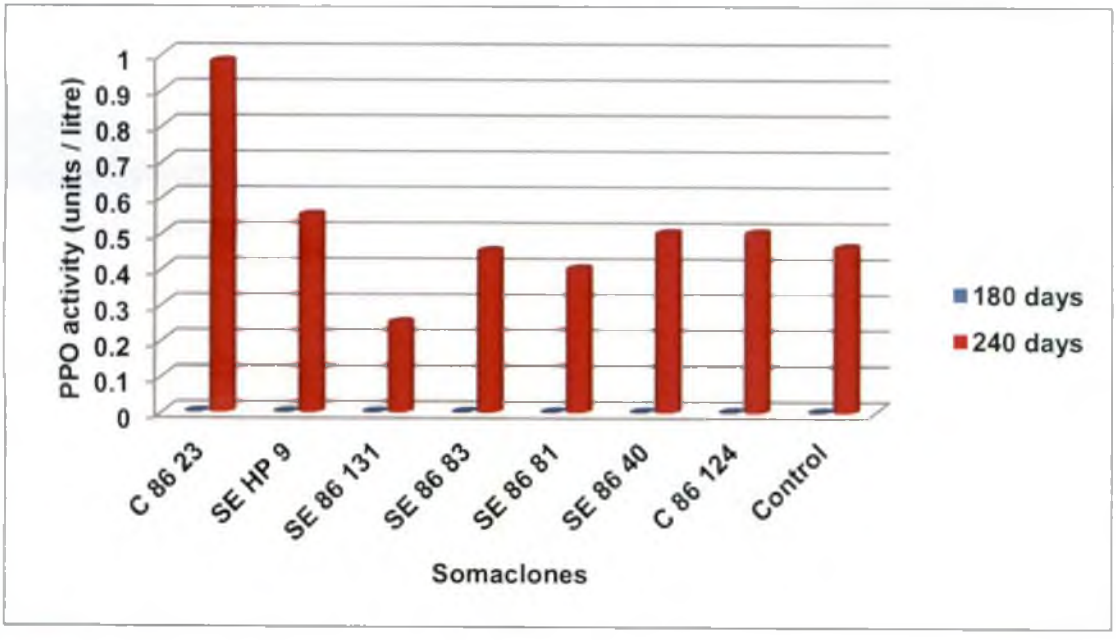


Fig 23. Polyphenol oxidase activity of ginger paste from different somaclones and control at initial and three months of storage

al. (2004) reported that addition of chemical preservatives and high processing temperature and pressure impairs protein synthesis causing the inactivation of intracellular enzymes leading to metabolic inactivation of microbes. Sivakumar (2013) observed a very slight increase of microbial load in amla candy during storage. The initial bacterial, fungi and yeast counts were $1x10^3$, $1x10^2$ and $1x10^2$ respectively, which had increased to $4x10^3$ bacterial counts, $3x10^2$ fungal count and $3x10^2$ yeast count at the end of the storage period. Lakshmi *et al.* (2015) reported similar results in ginger paste.

5.3.4 Sensory evaluation

For ginger candy and paste preparation, best somaclones were identified based on the results obtained from sensory evaluation. In ginger candy, among all the somaclones, SE 86 40 scored the highest total score (65.39) and was on par with control (64.98) and somaclone SE 86 131 (60.03) and in ginger paste, somaclone SE 86 131 scored highest (66.4) total score compared to other somaclones.

In both ginger candy and paste organoleptic scores showed a declining trend during storage. In ginger candy, among all the somaclones, SE 86 40 showed significantly higher total score (64.34) after 3 months of storage, followed by control (63.93) and minimum total score was in SE 86 83 (44.56), followed by somaclone C 86 23 (45.38). With respect to ginger paste, somaclone SE 86 131 had maximum total score (65.35) after 3 months of storage, followed by somaclone C 86 23 (63.3) and minimum total score was recorded in somaclone C 86 124 (37.95). Mir and Nath (1973) reported that overall acceptability of mango bar decreased during storage. Sivakumar (2013) reported that organoleptic scores of amla sweet candy slightly decreased with an increase in the storage period. The organoleptic scores of colour, appearance, texture, taste and overall acceptability were 8.9, 8.8, 8.6, 8.8 and 8.7 respectively at initial study period, which were reduced to 8.0, 7.9, 7.8, 8.0 and 7.9 for colour appearance, texture, taste and overall acceptability, respectively after nine

months. Increase of non enzymatic browning and polyphenol oxidase activity and build up of microbial population may have led to slight browning and decrease of colour. This might be responsible for decrease of organoleptic scores during storage in both ginger candy and paste.

The results revealed that somaclones SE 86 40 and SE 86 131 were highly suitable for the preparation of ginger candy and paste respectively, because these somaclones had lower crude fibre, higher volatile oil constituents, higher sensory scores than control and also showed lower variation in physico- chemical parameters during storage. Other somaclones such as SE HP 9, C 86 23 and SE 86 83 also can be considered suitable for value addition. Candy and paste from somaclones SE 86 40, SE 86 131, SE HP 9, control and SE 86 131, C 86 23, SE HP 9, SE 86 83 respectively, were under acceptable limits based on the scores, after three months of storage.

5.4 Conclusion

Evaluation of the 40 somaclones based on yield and quality attributes (driage, volatile oil, oleoresin, starch, fibre and chemoprofiling of volatile oil and non volatile pungent principles) revealed wide variability among somaclones, enabling good scope for selection. Quality analysis revealed that besides high volatile oil and oleoresin contents, somaclones exhibited low fibre content, which is a desirable attribute for quality ginger making them suitable as fresh ginger and for value added products. Screening for value added products indicated the exceptional suitability of SE 86 40 for ginger candy and SE 86 131 for ginger paste, whereas somaclones C 86 23 SE HP 9 and SE 86 83 can also be considered for value addition.

Based on evaluation for yield, quality attributes and value addition the following somaclones were selected for different end uses.

Table 18. Promising somaclones for different end uses

Attributes	Somaclones
Fresh ginger	SE 86 81, SE 86 131, SE 86 40, SE 86 83
Dry ginger	C 86 23, SE 86 81, SE 86 4, SE 86 41
Volatile oil extraction	SE 86 40, CHP 99
Oleoresin extraction	C 86 23, SE 86 81, SE 86 41
Gingerols extraction	SE 86 81, C 86 124
Candy preparation	SE 86 40, SE 86 131, SE HP 9
Paste preparation	SE 86 131, C 86 23, SE HP 9, SE 86 83

5.5 FUTURE LINE OF STUDY

1 Storage stability of the products for extended period.

2 Screening of more number of somaclones with low fibre and high flavour quality for value added products

SUMMARY

SUMMARY

Investigation on "Screening somaclones of ginger for value addition (*Zingiber officinale* Rosc.)" was carried out at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2013 to 2015. The study aimed at evaluation of somaclones of ginger for variability in quality attributes and identification of novel chemotypes through chemoprofiling and screening somaclones of ginger for product diversification and identification of elite types for value added products. The salient findings of the study are listed below.

EVALUATION OF SOMACLONES

Rhizome yield

- Somaclones exhibited wide variability in fresh and dry rhizome yield.
- Somaclones derived from Z-0-86, exhibited higher fresh rhizome and dry rhizome yield compared to other somaclones.
- Somaclones SE 86 81, SE 86 131, SE 86 83 and SE 86 40 showed higher fresh rhizome yield.
- Somaclones C 86 23, SE 86 81 and SE 86 41 showed higher dry rhizome yield.

Quality attributes

- Somaclones exhibited significant variations in quality attributes.
- Somaclones derived from Z-0-86, showed higher rhizome yield, driage, starch and oleoresin content compared to other somaclones.
- Somaclones derived from HP, showed lower crude fibre content and somaclones derived from Z-0-78, showed higher volatile oil content compared to other somaclones.

- Promising somaclones for oil and oleoresin extraction were SE 86 40, CHP 99 and C 86 23, SE 86 81 respectively.
- In general, the somaclones showed lower crude fibre content which is considered as a desirable quality attribute for fresh ginger and value added products.
- Somaclones with low crude fibre content were C 86 8, SE HP 8, SE HP 74, SE 86 83 and SE 86 131.
- Studies on changes in quality attributes of ginger somaclones with crop maturity indicated that essential oil and oleoresin contents decreased with increase in crop duration, while starch and crude fibre contents increased with crop maturity, in all the somaclones.
- Chemoprofiling of volatile oil of 11 ginger somaclones revealed the presence of 44 aroma compounds and some the compounds were unique to some somaclones.
- Identified unique compounds were β-citronellyl acetate, aromadendren, β – biasbolene, germacrene d, β-phellandrene, limonene, linalool and γ-cadinene.
- Out of 11 ginger somaclones, somaclone SE 86 131 showed highest volatile oil constituents (88.3%), followed by SE 86 40, SE 86 83 and SE 86 81
- Somaclone CHP 99 showed highest zingiberene followed by C 86 40, SE 86 40 and SE 86 131 indicating highly aromatic nature of somaclones.
- HPLC analysis of 12 ginger somaclones revealed presence of high gingerols and low shogaols contents in ginger rhizomes, which is an ideal character for good quality rhizomes.
- Out of 12 somaclones, somaclones C 86 124, SE 78 26 and SE 86 81 showed higher 6-gingerol content, the most biologically active pungent principle in ginger.

Screening somaclones for value addition

- Screening of seven somaclones of ginger for value addition revealed that somaclones SE 86 40, SE 86 131 are highly suitable for the preparation of ginger candy and paste respectively and other somaclones SE HP 9, C 86 23, SE 86 81 and SE 86 83 also can be considered for preparation of value added products.
- Storage study of ginger candy and paste revealed that there was no significant change in quality parameters even after three months of storage indicating acceptability of products on storage.
- Among seven somaclones screened, SE 86 131 showed least changes in quality of candy.
- The study could identify promising ginger somaclones for different end uses such as fresh ginger, dry ginger, volatile oil oleoresin and gingerol extraction and preparation of paste and candy as given below

Attributes	Promising somaclones
Fresh ginger	SE 86 81, SE 86 131, SE 86 40, SE 86 83
Dry ginger	C 86 23, SE 86 81, SE 86 4, SE 86 41
Volatile oil extraction	SE 86 40, CHP 99
Oleoresin extraction	C 86 23, SE 86 81, SE 86 41
Gingerols extraction	SE 86 81, C 86 124
Candy preparation	SE 86 40, SE 86 131, SE HP 9
Paste preparation	SE 86 131, C 86 23, SE HP 9, SE 86 83

- SE 86 81 can be considered as a multiple usage somaclone due to its suitability for fresh and dry rhizome, oleoresin and gingerols extraction. Somaclones SE 86 40 and SE 86 131 are suitable for fresh ginger and value added products.

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APPENDICES

APPENDICES

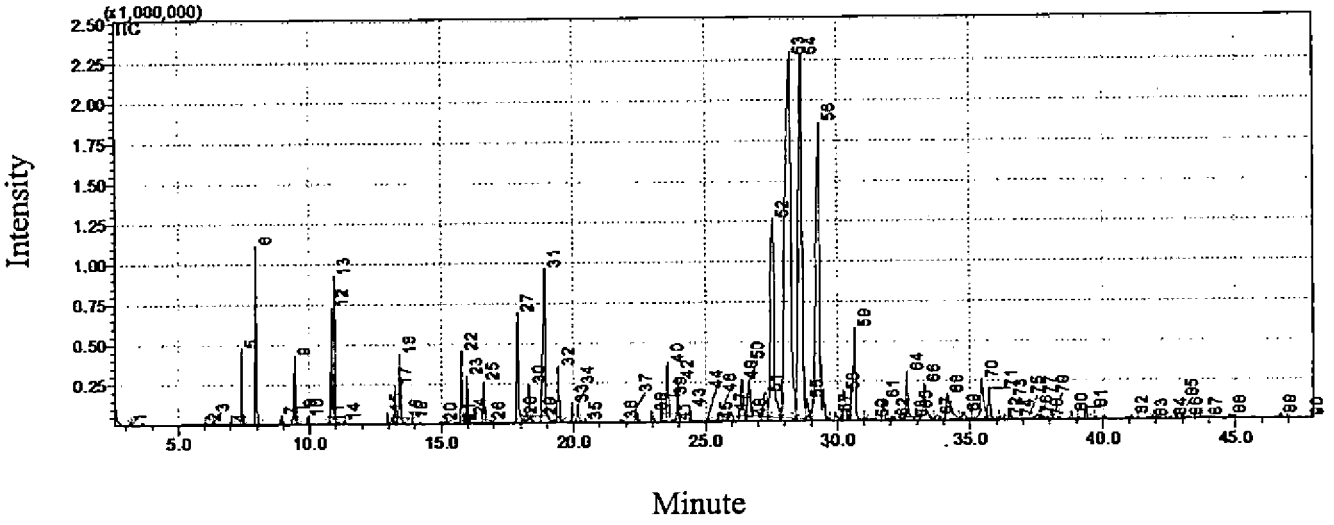

Fig 1. GC-MS chromatogram of somaclone SE 86 81

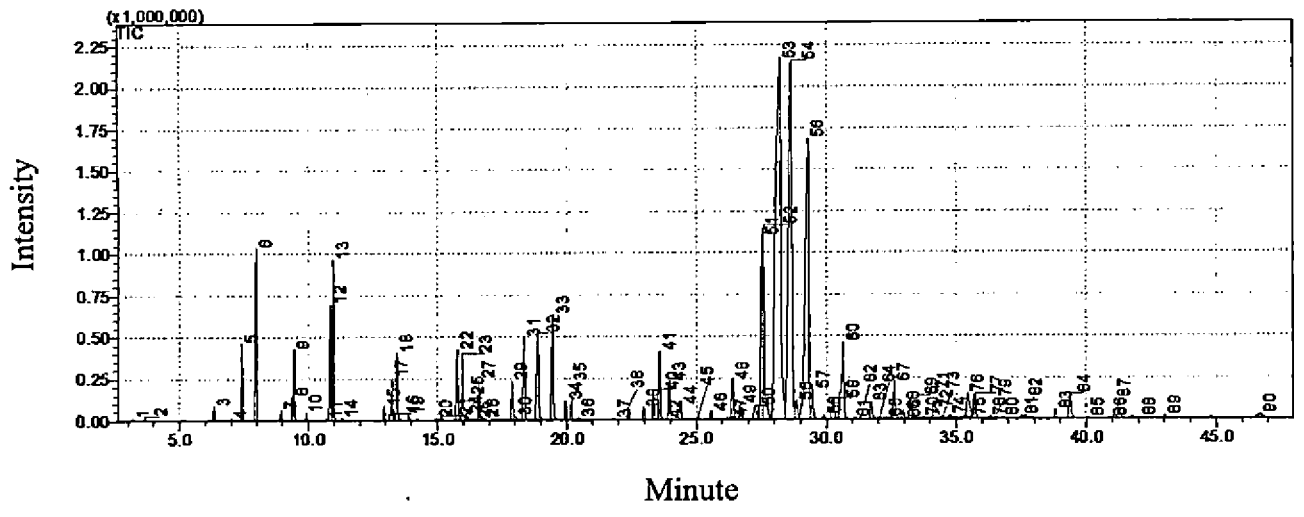

Fig 2. GC-MS chromatogram of somaclone SE 86 83

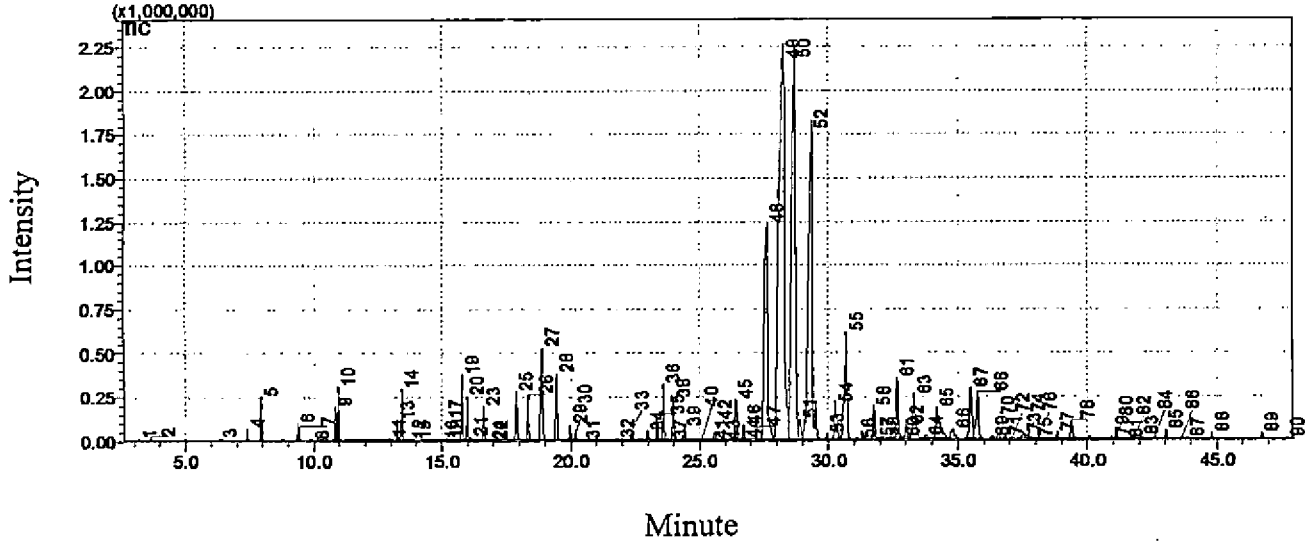

Fig 9. GC-MS chromatogram of somaclone C 86 23

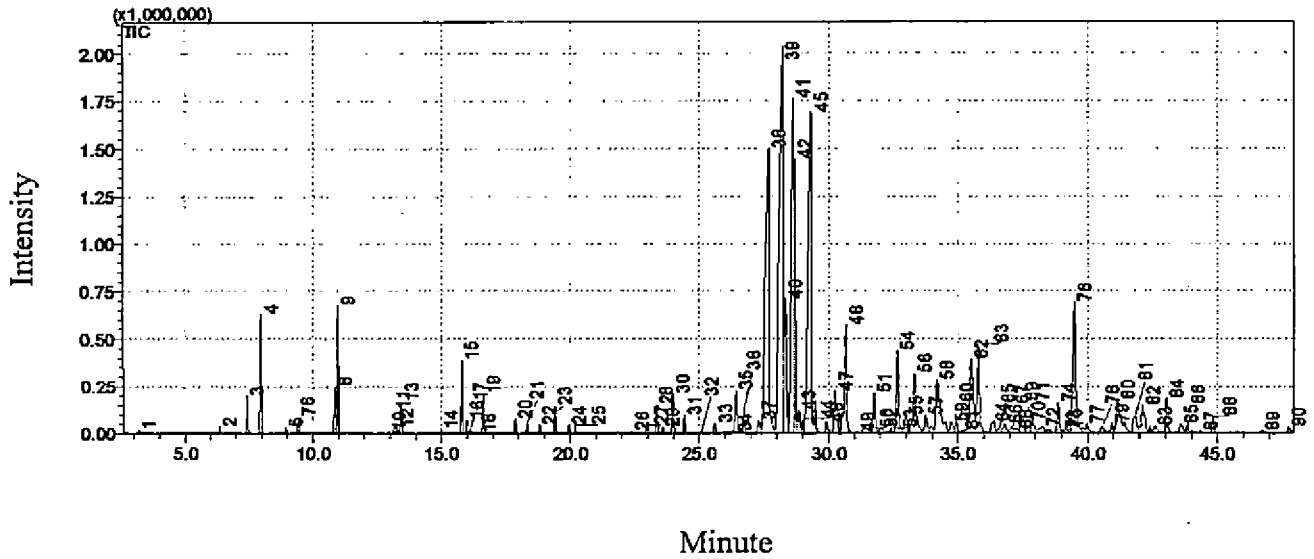

Fig 10. GC-MS chromatogram of somaclone C 86 124

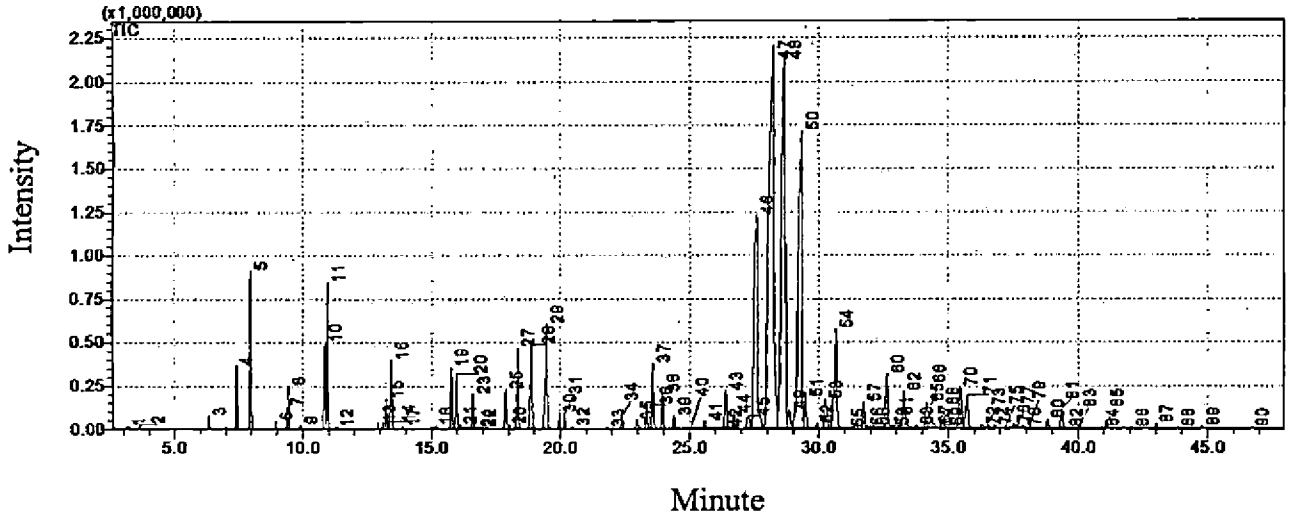

Fig 3. GC-MS chromatogram of somaclone SEHP 9

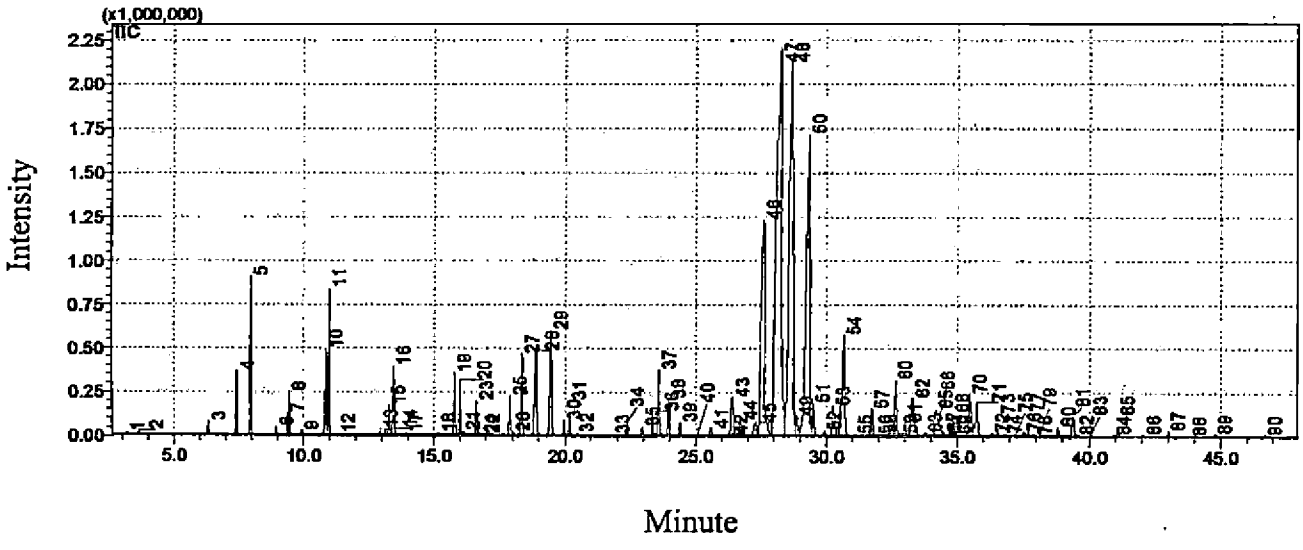

Fig 4. GC-MS chromatogram of somaclone SE 86 40

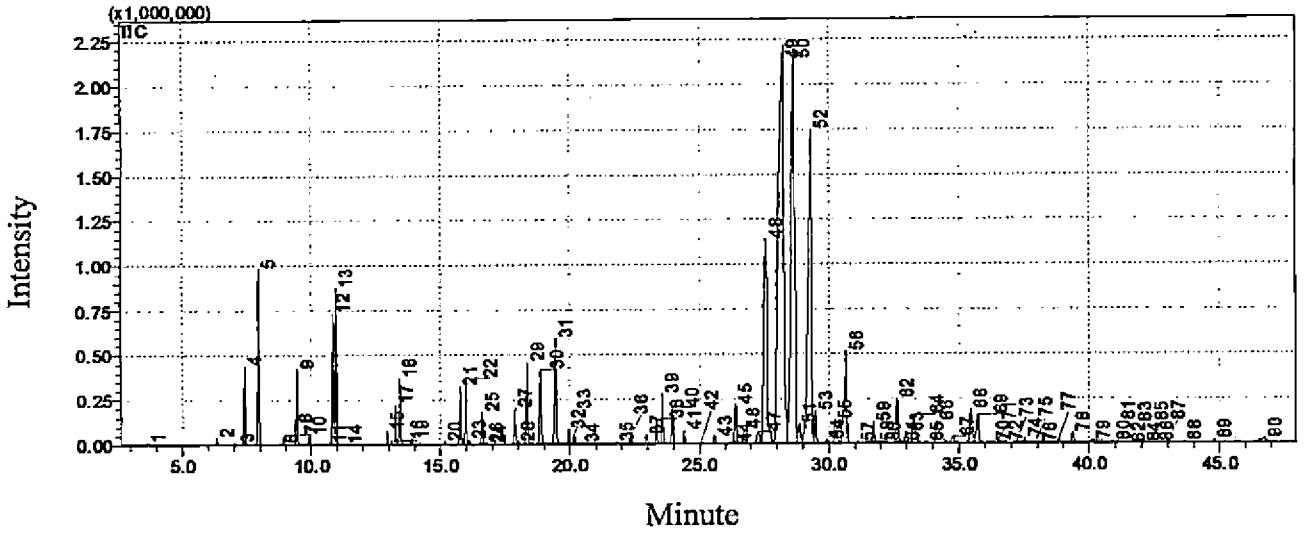

Fig 5. GC-MS chromatogram of somaclone SE 86 131

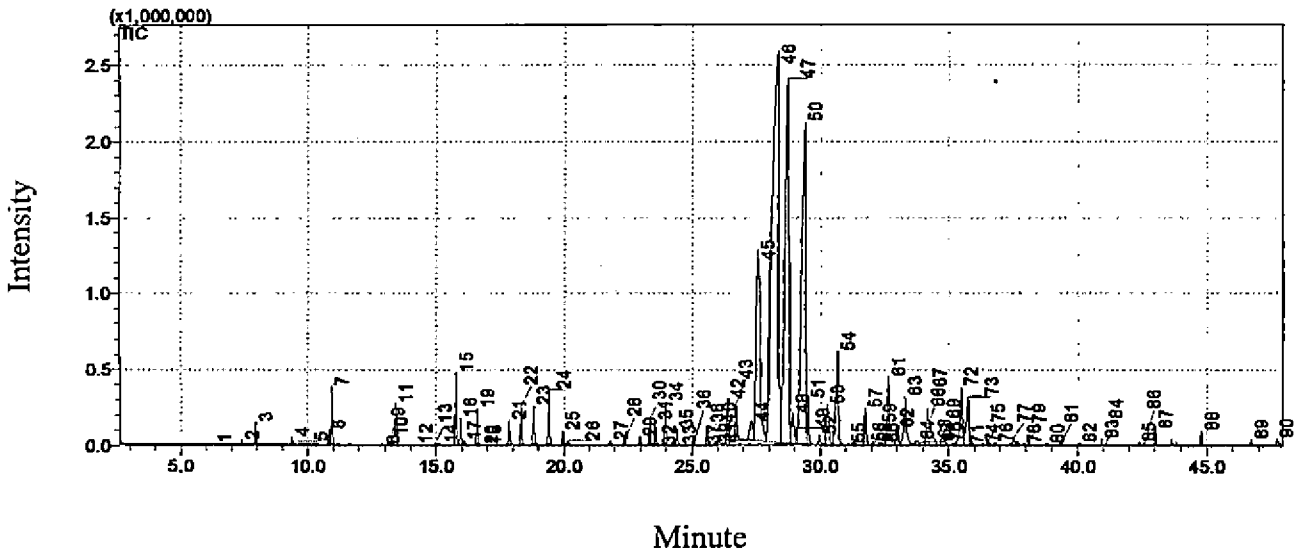

Fig 6. GC-MS chromatogram of somaclone C 86 40

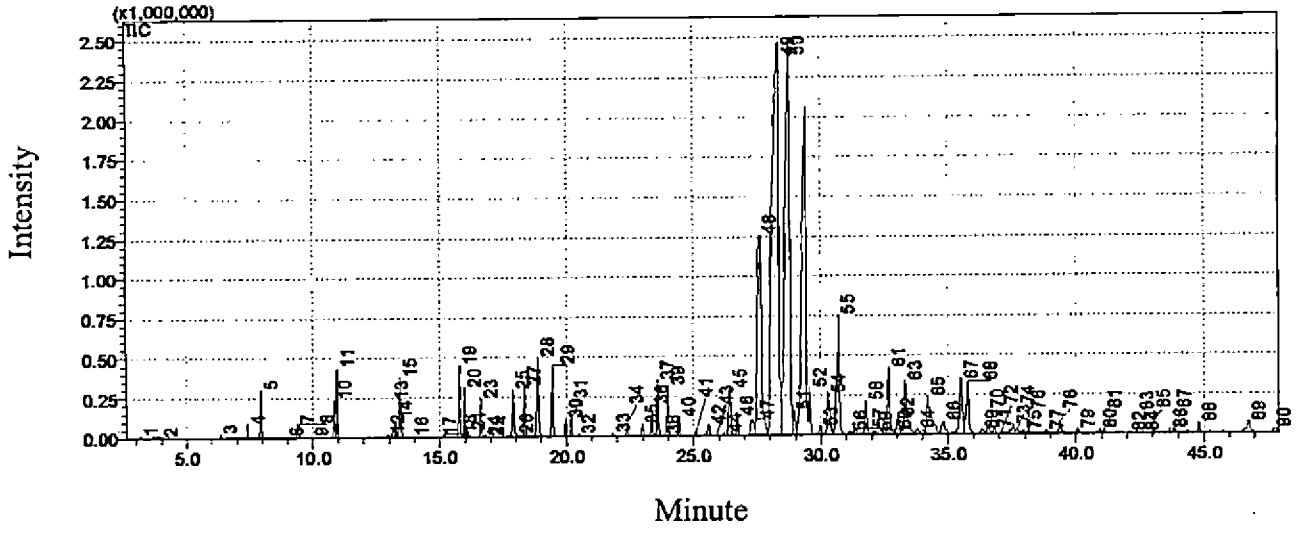

Fig 7. GC-MS chromatogram of somaclone CHP 99

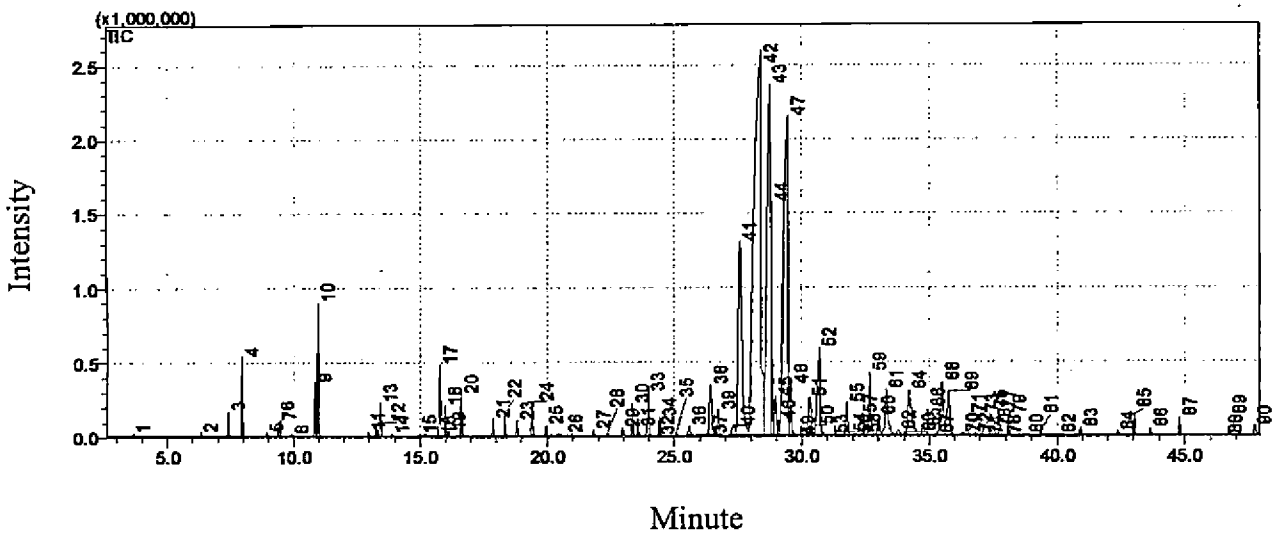

Fig 8. GC-MS chromatogram of somaclone C 86 139

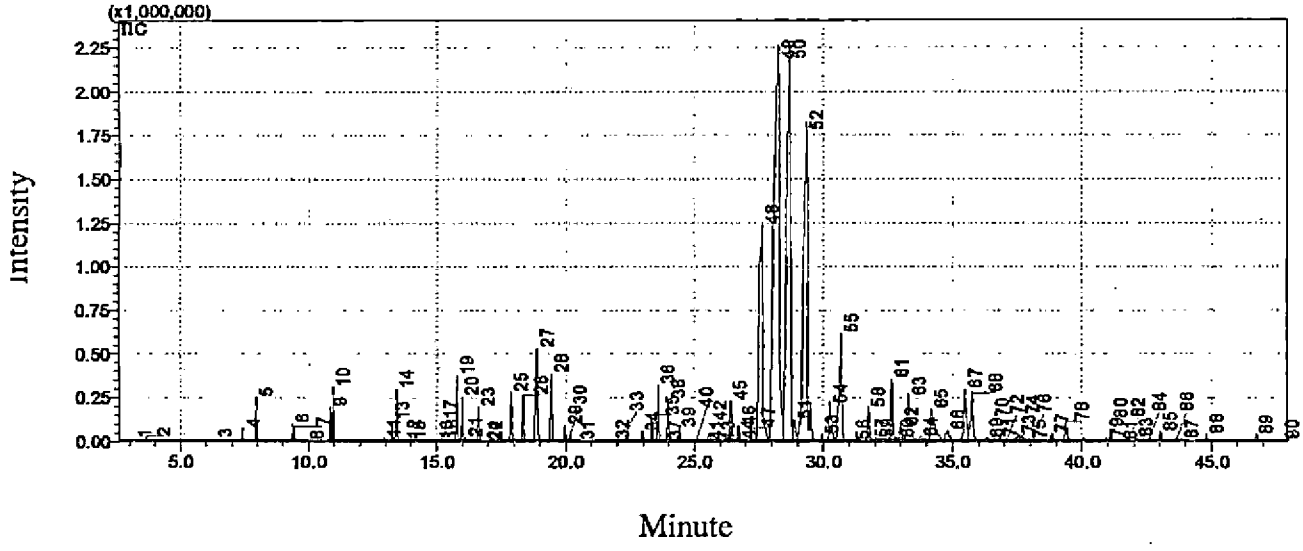

Fig 9. GC-MS chromatogram of somaclone C 86 23

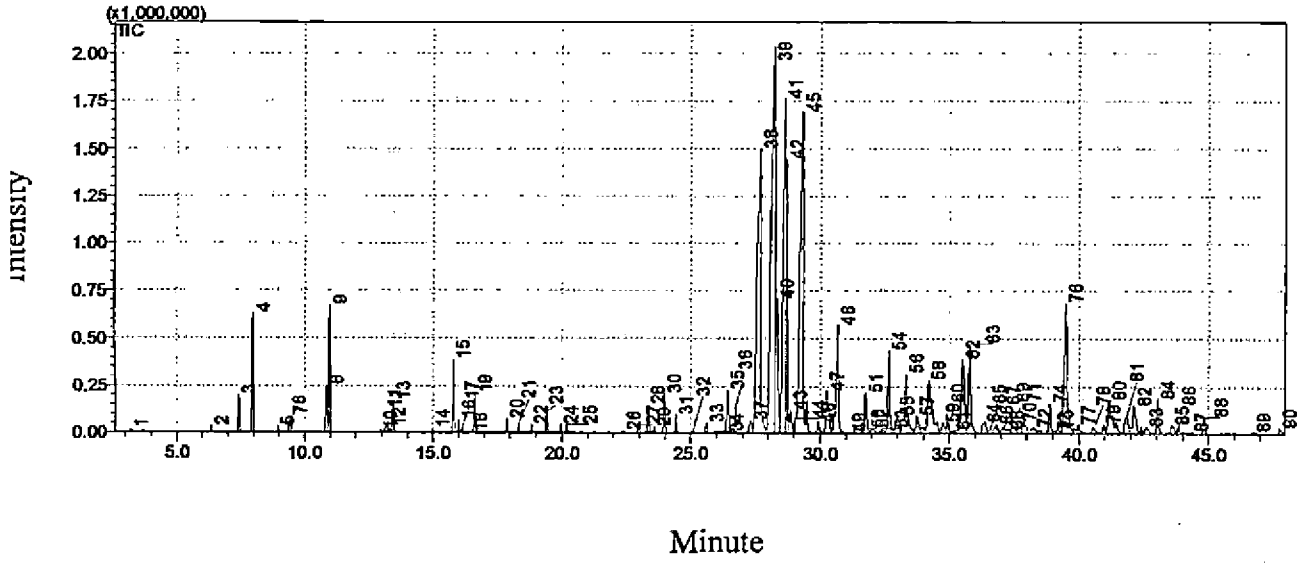

Fig 10. GC-MS chromatogram of somaclone C 86 124

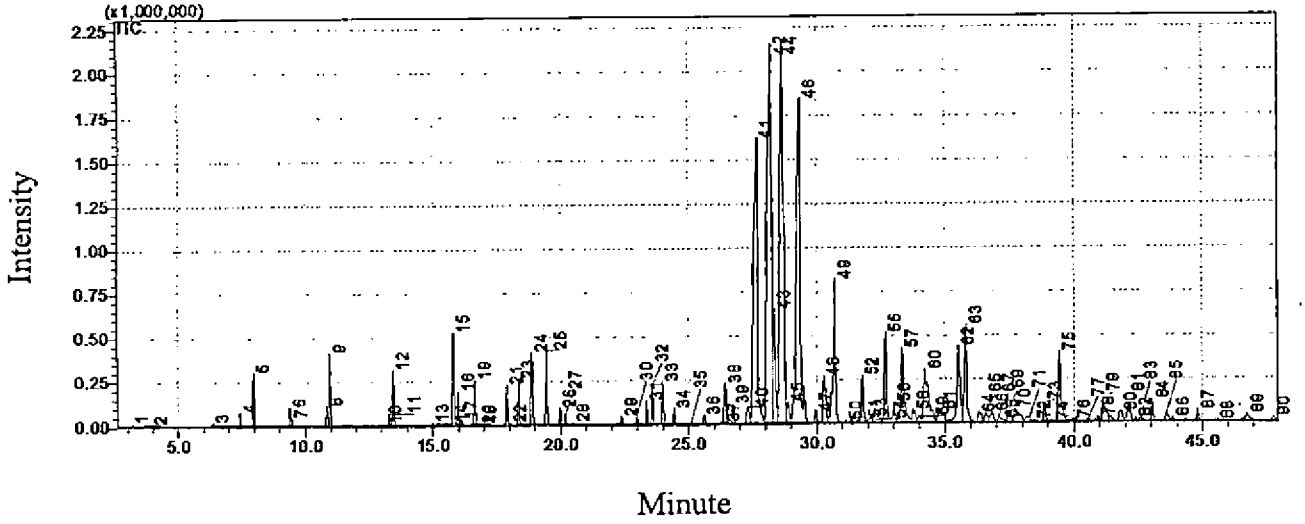

Fig 11. GC-MS chromatogram of somaclone C 86 32

SCREENING SOMACLONES OF GINGER (*Zingiber officinale* Rosc.) FOR VALUE ADDITION

By

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ABSTRACT OF THE THESIS

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DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

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ABSTRACT

The present study entitled "Screening somaclones of ginger (*Zingiber officinale* Rosc.) for value addition" was taken up at College of Horticulture, Vellanikkara during 2013-15 to evaluate forty somaclones for quality attributes and value addition. These forty somaclones were selected from a base population of 289 somaclones, developed through indirect organogenesis and indirect embryogenesis with and without mutagenesis from three cultivars (two induced polyploids Z-0-78, Z-0-86 and the diploid cultivar Himachal Pradesh) and maintained as a part of the DBT funded project at Dept. of Plantation Crops and Spices.

Somaclones exhibited wide variability in rhizome yield and quality attributes. Somaclones derived from polyploid parent Z-0-86 recorded higher fresh and dry rhizome yield compared to somaclones derived from Z-0-78 and HP. Among forty somaclones, SE 86 81 recorded highest fresh rhizome yield (28.81t ha^{-1}), followed by SE 86 131, SE 86 83 and SE 86 40. The somaclones C 86 23 showed highest dry rhizome yield (6.43 t ha^{-1}), followed by somaclones SE 86 81 and SE 86 41.

The quality attributes such as driage, volatile oil, oleoresin, starch and crude fibre contents varied significantly in the somaclones. Among forty somaclones, SE HP 8 recorded maximum driage (27.13%), followed by somaclones SE 86 41 and C 86 23. The somaclone SE 86 40 showed highest volatile oil content (2.40%), followed by CHP 99. Somaclones derived from Z-0-86 showed higher oleoresin content, with the highest value in C 86 124 (5.94%), followed by SE 86 41 and C 86 40. In general, the somaclones showed lower crude fibre content which is considered as a desirable quality attribute for fresh ginger and value added products. The starch content among the somaclones ranged from 40.59 to 52.56% and high starch yielding types were SE HP 8, C 86 139 and SE 78 26. Studies on changes in quality attributes of ginger somaclones with crop maturity indicated that essential oil and oleoresin

contents decreased with increase in crop duration, while starch and crude fibre contents increased with crop maturity, in all the somaclones.

Chemoprofiling of volatile oil of 11 ginger somaclones revealed the presence of 44 aroma compounds. Among the different classes of compounds, sesquiterpene hydrocarbons are quantitatively the major constituents. Among sesquiterpene hydrocarbons, zingiberene was predominant (23.28%) and the content was highest in the somaclone CHP 99 (29.64 %), followed by C 86 40 and SE 86 40.

HPLC analysis of pungency principles revealed that 6- gingerol was the predominant one in all the ginger somaclones. The highest 6-gingerol was observed in the somaclone C 86 124 (2.44 %), followed by SE 78 26 and SE 86 81. The content of total gingerols was also high in these somaclones. The shogaols level ranged from 0.24 to 0.49 % and highest shogaols content (0.49 %) was in the somaclone C 86 40, followed by SE 86 81 and C 86 124.

Screening of ginger somaclones for value added products such as paste and candy revealed that somaclones SE 86 40 and SE 86 131 were ideal for preparation of ginger candy and somaclones C 86 23 and SE 86 131, for ginger paste, because these somaclones recorded higher sensory scores than control and showed lower variation in physico- chemical parameters during storage. Studies on storage stability of the products revealed that parameters like moisture, colour, pH and sensory scores showed a decreasing trend, while TSS, titratable acidity, non enzymatic browning, polyphenol oxidase activity and microbial count showed slight increase in all somaclones and control.

The study could identify promising ginger somaclones for different end uses such as fresh ginger, dry ginger, volatile oil, oleoresin extraction and gingerol extraction and preparation of paste and candy.