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Effect of growth stages on the changes in bioactive compounds of Nagpur mandarin (*Citrus reticulata*) fruits of *Ambia* crops*

LALLAN RAM[1] and DINESH KUMAR[2]

National Research Centre For Citrus, Amravati Road, Nagpur, Maharashtra 440 010

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Citrus is one of the most important and widely grown fruit commodity (Talon and Gmitter 2008), classified as non-climacteric fruit with unique anatomical fruit structure (Spiegel-Roy and Goldschmidt 1996). Mandarin constitutes about 44% of total citrus production in which Nagpur mandarin (*Citrus reticulata* Blanco) rank first among mandarin group in central India. The fruit contains two peel tissues, the Flavedo accumulates pigments and compounds contributing to the fruit aroma, while the albedo comprises spongy cells rich in pectin. Fruits are known to be rich in varieties of bioactive compounds and nutrients which play a vital role in promotion of health (e g flavonoids, carotenoids and pectin) and prevention of chronic disease ((Liu 2003), both in developed and developing worlds. During the processing of citrus fruit for juice, peels are the primary byproduct. Citrus fruit development is characterized by changes in primary and secondary metabolites including sugars and citric acid being the major components of the juice sac cells. Sucrose is translocated to the fruits from the leaves throughout fruit development process and constitutes about 50% of the total soluble sugars (Ehud *et al*. 2011) which are of commercial importance, but very meager information is available about the bioactive compounds and nutrients synthesis at different fruit developmental stages of A*mbia* crops in sub-humid climatic condition of Central India. Therefore, this investigation was carried out with the objective to standardize the suitable growth stages for production of bio-compounds.

The present study was carried out during 2004–06 at experimental orchard of NRC for Citrus, Nagpur in 12-year old Nagpur mandarin budded on rough lemon rootstock. The fruits were tagged at petal fall (fruit set) stage for sampling at different stages, viz. stage-I marble stage, stage-II before colour turning stage(fully developed green colour), stage-III colour break stage and stage-IV mature stage. A completely randomized design (CRD) was followed (Panse and Sukhatme 1985) for each stages and replicated four times. A total of eighty fruits were subjected to analysis for various bioactive compounds in the fruits of *Ambia* crop. The total soluble solids (TSS) of juice were analyzed by using hand Refractometer (0–32% range), titratable acidity and ascorbic acid (AOAC, 1990), chlorophyll "a", "b" and total chlorophyll and Carotenoids (Wellburn 1994) using Dimethyl Sulphoxide (DMSO) as extractant, pectin in peel, juice, rag tissues (Rangana 2001), juice hesperidin and naringin(Hendrickson *et al* 1958, 1959), juice limonin (Wilson and Crutchfield 1968) were estimated. The peel oil was extracted by hydro distillation of fresh fruit peel in Clevenger's apparatus and was expressed in per cent oil content. The aldehyde content of refractive index and optical rotation of essential oil (Polax-2L-Atago Polarimeter) and colour by colour guard were analysed. Study revealed that mature *Ambia* fruits contained about 43.39% juice, 16.21% rag and 29.15% peel tissues, respectively contributes 45.4% processing waste which could be converted in to various value-added products (Table 1), indicating the high usefulness for processing industry specifically for development of value-added products (Ram and Singh 2007). The significant changes in Chlorophyll content was recorded during different developmental stages of Nagpur mandarin (Table 1). The cholorophyll 'a', chlorophyll 'b' and total chlorophyll content were recoded to be decreased from stage before colour break till maturation, but the loss of chlorophyll 'b' was found to be slower than chlorophyll 'a' with fruit maturity due to tight bound of chlorophyll 'b' with protein and less subject to enzymatic breakdown than chlorophyll 'a'. This indicated that the peel waste possibly be made available as a source of natural colour for food industry to replace the cultar dyes as has generally been used in food products (Mani 1999). The

[1,2] Senior Scientist (e mail: lallanram09@gmail.com, dineshsin@gmail.com)

Table 1 Physico-chemical changes in Nagpur mandarin fruits at different development stages

Physicochemical parameter/stage	Stage-I	Stage-II	Stage-III	Stage-IV	CD (*P*=0.05)
Fruit wt. (g)	18.74	99.26	106.33	114.14	10
Peel (%)	16.71	25.34	28.95	29.15	3
Rag (%)	18.65	20.20	24.32	16.21	2
Juice (%)	14.66	34.32	37.42	43.39	8
Chl a (mg/g fr.wt)	0.092	0.040	0.037	0.024	0.01
Chl b (mg/g fr.wt)	0.061	0.045	0.043	0.039	0.01
Total Chl (mg/g fr.wt)	0.152	0.085	0.081	0.063	0.01
Juice carotene (mg/gfr.wt)	0.005	0.063	0.025	0.039	0.02
Peel carotene (mg/gfr.wt)	0.085	0.103	0.172	0.423	0.12
TSS (%)	6.45	7.15	9.17	11.16	2.12
*p*H	2.54	3.78	3.51	3.59	0.20
Acidity (%)	6.46	1.37	0.90	0.69	0.22
Vit-C. (mg/100ml)	30.00	36.65	42.3	42.23	2.50

Stage-I, Marble size; Stage-II, before colour break; Stage-III, colour break; Stage-IV, mature fruit

carotene content in peel and juice tissues as described in table 1 showed the increasing trend during fruit developmental stage. Peel carotenoids was higher than juice carotenoids. The peel of mature fruits recorded the maximum of 0.423 mg/g fr.wt carotenoids on fresh weight basis. The accumulation trend of carotenoids in juice tissues were observed to be similar to those of peel carotenoids, indicating that consumption of fruits at mature stage possibly be highly useful as it is a source of Vitamin-A. The significant changes in juice, TSS, acidity and ascorbic acid were recorded during different fruit developmental stages (Table 1). The total soluble solids increased to 11.16% at fruits maturity stage which may be due to hydrolysis of acids and deposition of polysaccharides as has been reported in Kinnow by Ram *et al*. (2003). However, acidity decreased (0.69%) and Vitamin C increased (42.33 mg/100ml) in juice of mature fruits from marble stage. The *p*H of juice ranged between 2.54 and 3.59, respectively in fruits, showing that juice remains in acidic state (Table 1). Peel, rag and juice tissues were extracted for pectin content and expressed as calcium pectate (Table 2). Peel tissues contained higher pectin than rag and juice tissues. However, fruits showed the maximum of their peak for pectin during the colour development stage where insoluble proto pectin conversion took place into the soluble pectin (Yuan-Chuen *et al.* 2008). The mature fruits peel showed the maximum oil (0.75%) than those of marble stage fruits

Table 2 Pectin contents, peel oil and bio-flavines in Nagpur mandarin at fruit different developmental stages

Bioactive compounds	Stage-I	Stage-II	Stage-III	Stage-IV	CD (*P*=0.05)
Pectin in peel (%)	1.70	2.22	3.92	1.70	1.12
Pectin in rag (%)	0.20	3.91	2.60	1.17	0.35
Pectin in juice (%)	0.15	0.54	1.39	1.02	0.12
Oil content (%)	0.13	0.70	0.43	0.75	NS
Aldehyde (%)	0.00	1.88	0.63	0.93	0.12
Limonin (ppm)	5.75	6.41	6.46	5.28	NS
Naringin (ppm)	111.6	110	56.34	62.5	13
Hesperidin (ppm)	17.91	36.0	92.37	51.43	11

Stage-I, Marble size; Stage-II, before colour break; Stage-III, colour break; Stage-IV, mature fruit

(Table 2). This variation in oil was possibly because of underdeveloped oil glands at early fruits developmental stages. The maximum aldehyde content (1.88%) of peel oil was in fruits at before colour turning indicating the good quality oil. The naringin and hesperidin are known as flavonoids (Table 2). The fruits sampled at mature stage recorded the lower juice limonin (5.28 ppm) and higher juice naringin (110 ppm) in the fruits at stages before colour turning. Limonin and naringin decreased in similar pattern. On the other hand, not much variation in juice hesperidin was recorded. Hence, it can be inferred that several bioactive compounds are present in the Nagpur mandarin *ambia* crop at different fruit developmental stages which could possibly impart their characteristics on processed product and also provide evidence base that help in fighting against malnutrition and health promotion.

SUMMARY

The fruits of Nagpur Mandarin *Ambia* crops were analyzed for the changes in bio-active compounds. About 45.4% processing waste as peel, rag and seed can be used for byproduct development. However, TSS increased gradually from 6.45% (marble) to 11.16% (mature fruits).While, acidity dwindles at mature stages (0.69%). The *p*H of juice ranged between 2.54 and 3.59, indicating that juice remains in acidic state throughout the maturation period. The juice and peel tissues of mature fruits had higher carotene of 0.039 mg/gfr.wt. and 0.423 mg/g fr.wt than that of juice tissue. The pectin in juice ranged from 0.15 to 1.03 mg/g fr.wt during maturation. Peel oil and oil aldehyde content was recorded higher of 0.75% and 0.93% in the fruits harvested at mature stage. The juice limonin decreased to 5.28 ppm and naringin increased to 110 ppm in the mature stage in *ambia* crops.

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