Chemical Composition Change in Pineapple during Ripening: the Optimum Stage for Drying

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ABSTRACT

In order to determine the optimum stage to carry out drying experiments of pineapple, its composition change was studied during the ripening. Since degradation of important nutrient such as Vitamin C (L-ascorbic acid) and loss of volatile compounds can occur during drying, they were determined at different ripening stages. As the ripening stage of fruits is characterized by its soluble solids and titrable acidity, they were also measured. After harvested, the fruits were stored and the ambient conditions were registered until the 20th day after harvest. The results showed the influence of the ripening stage in the pineapple composition. Due to biochemical reactions that occur during this period, the composition changed in quantity. The riper the fruits, the higher the number of volatile compounds and their amount. The variation of L-ascorbic acid content did not show a clear trend as the one observed for the volatiles compounds.

Keywords: Aroma, vitamin C, ascorbic acid, chemical composition, drying of fruits, solid phase micro extraction (SPME), Brazil

1. INTRODUCTION

During ripening of fruits and vegetables, their chemical composition change abruptly due to the formation of volatile compounds, the variation of their physical chemistry properties and others. The presence of some components is of great importance for the acceptance of consumers and should be considered before exposing them to industrial processes. Drying is a very common process applied to food systems. Most of drying techniques exposes the product to high temperatures for a long time. Because of that, degradation of important nutrient such as Vitamin C (L-ascorbic acid) and loss of volatile compounds can occur.
Vitamin C (L-ascorbic acid) is an important nutrient for humans. Among its physiological properties, it plays a role as a biological antioxidant. However, L-ascorbic acid is a very sensible compound and usually is easily degraded during drying processes. Its stability depends on

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temperature, pH, presence of oxygen, etc. Since this nutrient tends to degrade during drying processes, it is usually taken as a quality parameter of the process.

Food flavor and aroma are other properties that influence the product acceptance. The aroma of fruits is composed of an exclusive combination of volatile compounds. The different proportions of the volatile components and the presence or absence of trace components often determine aroma properties (Lavid et al, 2002). A mixture of volatile components may be presented in a single fruit, but only a specific volatile component or group of components contribute for the unique aroma of fruit. Such components are often present in small amounts and their presence is a significant factor influencing the public's food-buying decisions and its perception of food quality (Sunthonvit et al, 2007).

In general, fruits composition changes after harvesting and during storage. The typical flavor of a fruit is developed during ripening. Esters are generated by the esterification of alcohols and acyl-CoAs, catalyzed by alcohol acyltransferases. Substrates for this esterification are thought to derive primarily from both fatty acid and amino acid metabolism (Sanz et al., 1997). Fatty acids serve as ester precursors, catabolized through two major pathways, b-oxidation and the lipoxygenase system. Fruits produce acetaldehyde and ethanol during maturation and ripening (Speirs et al., 1998). Pyruvate decarboxylase and alcohol dehydrogenase (ADH) are two important enzymes responsible for acetaldehyde and ethanol production, respectively. ADH has been implicated in the response of plants to stress, and is responsible for ethanol production under anaerobic conditions; however, ADH genes are also expressed in plant tissues in a developmentally regulated manner, particularly during fruit ripening (Manríquez et al., 2006).

The change of chemical composition of fruits and vegetables along their ripening has been studied by some authors. The Vitamin C content of white guava (Soares et al., 2007), yellow bell pepper (Antoniali et al., 2007) and sweet peppers (Martinez et al., 2005) were determined at different ripening stages. For those products, they reported an increase of the vitamin content during the ripening. On the other hand, Marques et al. (2007) reported the lowest vitamin C content in ripe samples of acerola.

With the objective of exploring the evolution of volatile compounds during berry development, Kalua and Boss (2009) focused grape and wine aroma research on the raw material and studied its volatile compound evolution from early berry development to late ripening. Solid-Phase Microextraction-Gas Chromatography-Mass Spectrometry (SPME-GC- MS) was used to analyze volatile compounds. The study showed that grapes release different compounds at different stages of berry development. It is apparent that grape berries are rich in volatile compounds during early development and that the evolution of volatile compounds changes during development. The progress of volatile compounds has shown that esters are characteristic of early berry development with aldehydes dominating the midberry developmental stages and finally alcohols appearing during late berry development.

The formation of aroma volatile compounds was assessed during maturation of 'Pink Lady' apples (Villatoro et al., 2007). Low production of aroma volatiles was observed in early harvested fruit, which gradually increased as ripeness approached. Hexyl acetate, hexyl 2-methylbutanoate, hexyl hexanoate, hexyl butanoate, 2-methylbutyl acetate and butyl acetate were prominent within the blend of volatiles produced by fruit throughout maturation. Multivariate analysis showed these compounds had the highest influence on differentiation of maturity stages, indicating that aroma volatile emission is an important factor for definition of fruit ripeness, which suggests production of these esters might be useful as an index of maturity.

The physicochemical properties of fruits and vegetables can influence their acceptance by the consumers. The evolution of components formation during ripening can be explored so as to identify when biosynthetic pathways may be targeted to achieve a greater appreciation of fruit and their processed products. Thus, the objective of this work was to study the change of volatile and L-ascorbic acid contents during pineapple ripening. Pineapple was chosen since it is an exotic tropical fruit with a large production in Brazil. The results obtained may provide substantial information for food processing techniques that exposes the product to heat, such as drying.

2. MATERIALS AND METHODS

In order to study the change on the composition of pineapple (Smooth Cayenne, Ananas comosus L. Merr) during the ripening, unripe fruits (30 units) were purchased directly from the producer (CEASA, Campinas, SP, Brazil). The fruits were stored and analyzed until the the 20^{th} day after harvest. During that period, the environment conditions (temperature and relative humidity) were recorded using a thermo hygrometer (Betha Eletrônica, Ummi model, Brazil) connected to a computer. The soluble solids content, the titrable acidity and the moisture of the fruits were measured in order to characterize the ripening stage. The moisture content of the samples was determined using a vacuum oven (70 ºC, 15 kPa, 24 h). Soluble solids were determined using a Q1 refractometer (Atago, Japan) and the titrable acidity was determined using NaOH solution (0.1N) and phenolphthalein (AOAC, 1995). The shell color was analyzed visually, dividing the pineapple into quarters and observing the percentage of parts green, yellow and orange. L-Ascorbic acid and volatile compounds were determined by liquid chromatography (HPLC) and dynamic headspace solid phase microextraction (DHS-SPME) coupled to gas chromatographer-mass spectrometer (GC-MS), respectively. Only the central part of each fruit was used to characterize the fruits since their composition may vary from the top to the base.

2.1 L-Ascorbic Acid Determination

Pineapple samples were weighed and blended for 45s with addition of 100ml of buffer solution (KH_2PO_4 0.01M, pH=2.59). This slurry was filtered and portions were injected into a high liquid performance chromatographer (HPLC) using the same buffer solution as a mobile phase. Chromatographic conditions were: C18 column at ambient temperature, mobile phase at 0.5ml/min and detection at 250nm. A calibration curve was plotted using a standard L-ascorbic acid solution.

2.2 Volatiles extraction and identification

Manual headspace Solid Phase Micro Extraction (SPME) coupled to Gas Chromatography-mass spectrometry (GC-MS) was used for the qualitative analysis of fresh pineapples volatile composition. This technique has been widely used for the extraction and pre-concentration of flavors and fragrances samples due to its simplicity of manipulation, that avoids thermal decomposition, oxidation, photolysis and others undesirable processes. SPME does not use solvent, has high power of concentration, is applicable to many samples and facilitate the

transport of extracted material to the gas chromatographer (Augusto et al., 2003). Samples of pineapple were homogenized in a blender with pieces of dry ice aid to prevent loss of volatile compounds. In each extraction, 1.50 g of chopped pineapple sample was transferred to the flask of DHS-SPME system. The system consisted of a 5.0 mL conventional septum-sealable glass vial, closed by a labmade Teflon piece that allows flushing of the vial with a stream of gas. The gas flowing from the vial, carrying volatilizable species from the sample passes by a glass tube (50 mm length x 0.75 mm i.d. x 5 mm e.d.), where SPME fiber can be inserted to collect the analytes. The purging gas was air from FIA peristaltic pump. The system was kept at 60ºC. It was allowed 5 min to equilibrium between sample and headspace and then the SPME fiber was exposed to the purging gas during 15 min. A 75 μm Carboxen-polydimethylsiloxane (PDMS) fiber was used for the extraction procedure. The gas chromatography analysis parameters are presented in Table 1. Peak identification was performed using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) version 2.63 and the NIST Mass Spectral Search Program v. 1.7. Identification of the detected species was performed by comparison with the mass spectra library.

Table 1. GC analysis parameters for volatiles extraction of pineapple

Apparatus	Hewlett-Packard GC 5870-MSD HP5890-II
Column	HP-5MS (30 m x 0.25 mm, 0.25 μm)
Carrier gas	Helium 5.0
Mode	Splitless
Injector temperature	270ºC
Detector temperature	280°C
Oven temperature	Initial: 40°C
	40°C $\rightarrow$ 150°C, 3°C. min^{-1}
	150°C $\rightarrow$ 240°C, 20°C.min^{-1}

The variation of volatile content of pineapple during maturation was evaluated using the total area, which corresponds to the total peaks area of chromatograms patterns.

3. RESULTS AND DISCUSSION

3.1 General characteristics

The fruits were characterized by the shell color, soluble solids content, acidity, moisture content, L-ascorbic acid and volatile composition.

The fruits were stored at ambient conditions and the temperature and relative humidity were recorded during that period (Figure 1). Figure 1 shows the average temperature and relative humidity (confidence interval 95%) for each day of storage. The pineapples were purchased and stored in the laboratory on the second day after harvest. A small oscillation in the ambient temperature and a more significant variation in the relative humidity can be observed during that period.

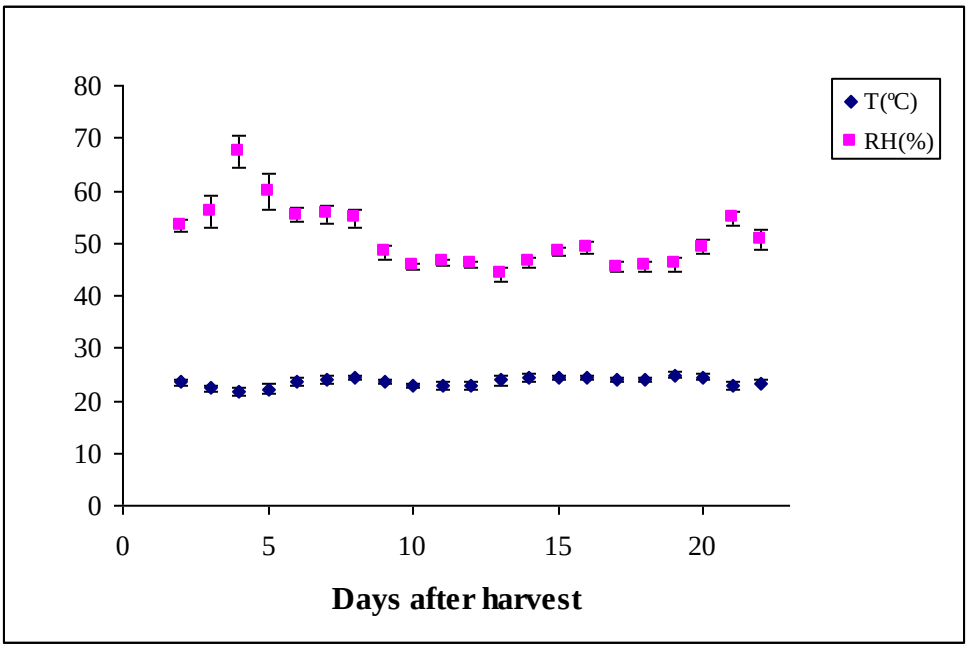

Figure 1. Average temperature and relative humidity during the period of fruits storage.

The fruit characterization was performed in between the 4rd and 20th days after harvest. The shell color, soluble solids and moisture content, acidity, L-ascorbic acid and the volatile composition of pineapple fruits were analyzed. The data obtained for shell color, soluble solids content, moisture and acidity are presented in Table 2. The acidity of the product is expressed as grams of citric acid per 100 grams of fresh fruit. It can be noted in Table 2 that the moisture content (wet basis) of fruit pulp showed no significant difference during the ripening process. Because of some biochemical reactions that occur during ripening, it was expected an increase for the soluble solids content in the advanced stages of maturation, a phenomenon that was observed, but at low intensity.

Table 2. Biochemical changes of *Smooth Cayenne* pineapple during ripening

Days after harvest	*Acidity (g/100g)*	*Soluble solids S.S. (°Brix)*	*S.S./Acidity*	*Moisture (%)*	*Shell color*
4	0.603	6	9.95	90.81	Green
6	0.617	6	9.72	91.39	0.75 green - 0.25 yellow
8	0.597	6	10.05	92.20	0.75 green - 0.25 yellow
11	0.699	6.5	9.30	90.94	0.5 green - 0.5 yellow
13	1.160	8.5	7.33	88.30	0.5 green - 0.5 yellow
15	1.167	8.5	7.28	89.08	0.8 yellow - 0.2 green
20	1.112	9	8.09	89.08	0.7 orange - 0.3 yellow

3.2 L-Ascorbic Acid Determination

Figure 2 presents the L-ascorbic acid content of pineapple fruits at different ripeness stages. In order to compare the results obtained, a brief literature search was done, but no results for L-

ascorbic acid change in pineapple during ripening were found. As commented earlier, the L-ascorbic acid content of other fruits and vegetables during ripening was investigated by different authors. For white guava (Soares et al., 2007), yellow bell pepper (Antoniali et al., 2007) and sweet peppers (Martinez et al., 2005), an increase of the vitamin content was observed during the ripening. On the other hand, Marques et al. (2007) reported the lowest vitamin C content in ripe samples of acerola.

Ishak et al. (2005) determined the ascorbic acid content of ambarella at three different ripening stages. They did not observe a clear trend for the variation of that nutrient during the ripening. The values obtained for the fruits varied between 4.65 to 5.86 mg/100g.

It is not possible to observe clear trend for the L-ascorbic acid in pineapples samples during the ripening too. The absolute values obtained are in good agreement with some reported in the literature. The reference value used by the United States Department of Agriculture (USDA) is 16.9 mg/100g (the ripening stage is not informed). The L-ascorbic acid content of the central part of the fruits at different ripening stages of the present study varied from 15-20 mg/100g.

Ramallo and Mascheroni (2004) also determined the ascorbic acid content of pineapple. They analyzed different portions of the fruit and the contents varied from 7 to 19 mg/100g. The ascorbic acid content of the central part of the fruit was approximately 14 mg/100g.

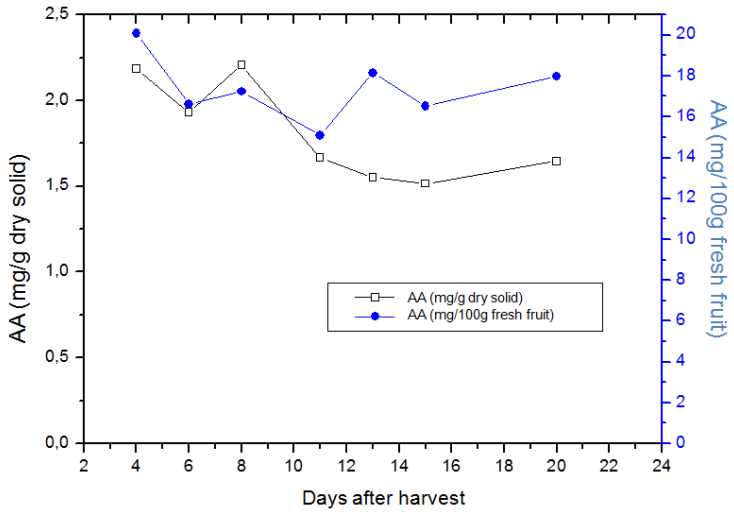

Figure 2. L-ascorbic acid content in pineapple at different ripening stages

3.3 Volatiles extraction and identification

The volatile composition of *Smooth Cayenne* pineapple was studied and, as Figure 3 shows, the volatile content abruptly increased during ripening, especially between the 15th and 20th days.

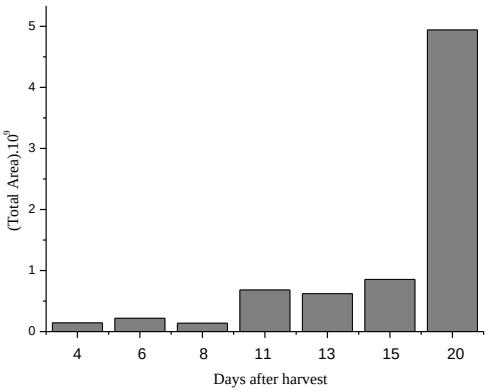

Figure 3. Total volatile content of fresh pineapple along maturation.

It can be noted that the volatile composition became much richer as days passed by. Figure 3 shows a large increase in total pineapple composition throughout maturation. This raise was already expected due to the formation of volatile compounds. It was observed mainly the formation of esters and terpenes. In addition, Figure 4 shows the chromatograms pattern of pineapple completely green (a) and completely mature (b). For the first sample, it was verified a presence of few volatiles, as the methyl hexanoate (8.501 minutes of retention time), presented as the most abundant compound (36.70%), followed by methyl 3–(methylthio)-propanoate (11.44%). On the other hand, the chromatogram of the last sample, (70% of shell orange), had much higher volatile composition content, as shown in Figure 4(b). The methyl hexanoate (26.94%) was the most abundant, followed by methyl 3–(methylthio)–propanoate (12.80%), methyl octanoate (11.65%) and ethyl octanoate (11.13%).

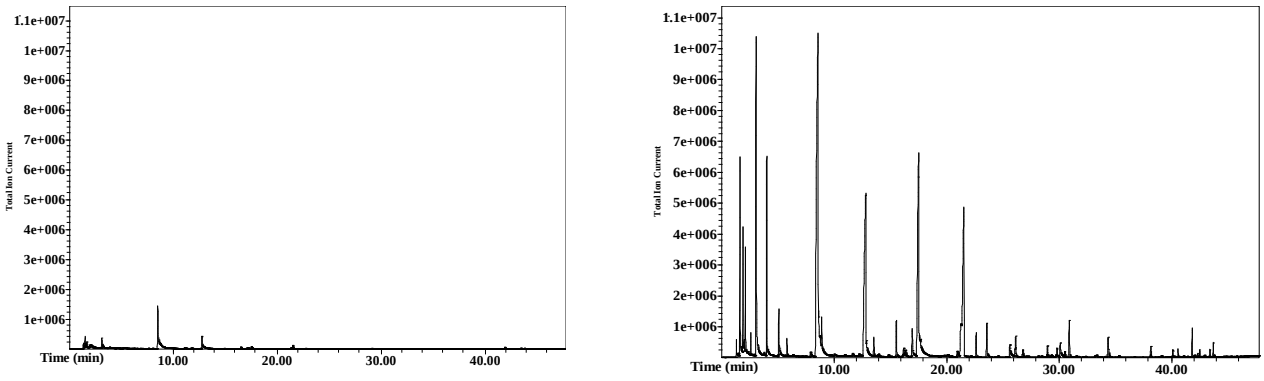

Figure 4. Chromatogram patterns obtained for pineapple shell totally green (a) and 70% orange-30%-yellow (b).

According to Engel et al. (1990), the pineapple volatile content is essentially formed by esters, aldeydes and alcohols. And among these components, methyl butanoate, methyl 2-methyl-butanoate and methyl acetate are considered some of the most important for the pineapple aroma (Tokimoto et al., 2005). Figure 5 shows that these compounds were in a very low amount at the begging of the ripening process, and their concentration was getting higher as days passed.

Figure 4 shows that there was formation of many compounds during ripening, especially esters. During the ripening of melon, Shalit et al. (2001) determined the activity of acetyl coenzyme A, which is the enzyme that catalyzes the formation of esters from the alcohols alcohols acetylation. It was shown that its activity increases during ripening, which is linked with increased concentration of soluble solids. Sulphur esters, almost not present in green sample, appeared in large quantities in mature samples. According to Ardö (2006), thioesters are formed from catabolism of amino acids.

Throughout the ripening process, it was observed a gradual change in shell color, initially totally green until achieve 70% of color skin in orange. This change in color was accompanied by an increase in the concentration of soluble solids with increasing volatile composition. The study shows that the degree with richest volatile composition was the last sample (20 days after harvest), with 70% of the shell in orange and 30% yellow. Also, this degree presented higher concentration of soluble solids.

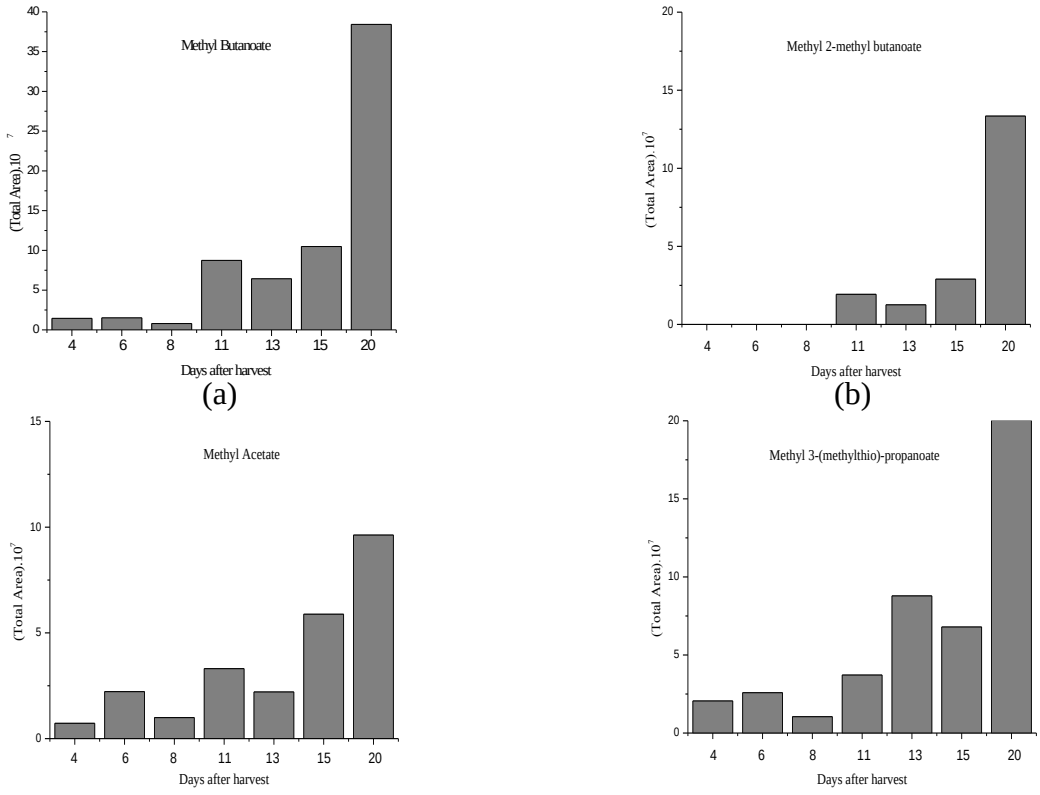

Figure 5. Variation of peak area for methyl butanoate (a), methyl 2-butanoate (b), methyl acetate (c) and methyl 3-(methylthio)-propanoate (d) in pineapple along maturation.

4. CONCLUSIONS

The study of pineapple composition during the ripening process showed a strong influence of the maturation degree on the fruits characteristics. The shell color changed considerably as well as the acidity, solid soluble content and volatile composition. Due to biochemical reactions that occur during this period, the volatile composition increased in quantity. The riper the fruits, the higher the number of volatile compounds and their amount. It was clearly observed that important components for pineapple aroma were formed during this study. The variation of L-ascorbic acid content did not show a clear trend as the one observed for the volatiles compounds.

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