Chapter 3

Materials and Methods

The study on postharvest physical and biochemical changes of 'Keaw Morakot' mango was divided into 3 experiments as follows:

Experiment 1 Changes of quality and enzyme activity during ripening of 'Keaw Morakot' mango fruit at ambient condition

'Keaw Morakot' mango fruit were harvested from a local commercial orchard in Baan Hong district, Lamphun province. The fruit were transported to the laboratory of the Department of Postharvest Technology, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai. Upon arrival, the fruit were dipped in 100 ppm sodium hypochlorite for 5 minutes. After dipping, the fruit were floated in water in order to select the fruit which only sink in the water for using in this experiment. The fruit which floated in water were discarded. After that, the fruit which sank in water were sorted into 3 maturity levels, by floating in 3 concentrations of brine solution, 4, 6 and 8% (specific gravity 1.04, 1.06 and 1.08, respectively), as Maturity 1, Maturity 2 and Maturity 3. Then, the fruit were stored at ambient condition (26-31˚C, 60-70% RH) to ripen, and analyzed for qualities everyday for 7 days. The panel test was evaluated only on day 7.

The experiment was arranged in a completely randomized design (CRD) with three treatments. All treatments were replicated three times.The data were analyzed by performing analysis of variance, using SX-statistic program. Mean comparison was done by the least significant difference test (LSD) at $\rho = 0.05$. The parameters were examined as follows:

1. Physical changes of pulp and peel

1.1 Firmness

Principle

Generally, texture is a group of physical properties that derives from the structure of the fruit, is sensed by the feeling of touch and related to the deformation and disintegration of the fruit. The fruit texture is a consequence of microstructure, which in turn may be affected by both chemical composition and physical forces. Texture studies are now firmly based on established

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principles of rheology and the measurement of mechanical properties of fruit which cover all the basic aspects of the science of rheology. Force measurement insruments are the most common equipment for objective texture measurement. Force has the dimensions mass x length x time^{-2}. The standard unit of force is the Newton (N) (Askar and Treptow, 1993).

Determination

The firmness of mango pulp was measured after peeling the fruit and measurements were taken at three different positions on the fruit by Texture analyzer TA-XT2 (Stable Micro System Ltd., Haslemere, Surrey, UK), using a flat plunger of 5 mm of diameter which penetrated deep to 10 mm and the moving rate was 100 mm/minute. The value was recorded as N.

1.2 Color changes of peel and pulp

Principle

The color of fruit is undoubtedly the first and the most important part of its quality attribute. It is often regarded as an index of general quality determination. The color can be measured by reflection from the surface of the fruit. When a photoelectric cell replaces the eye, thus largely eliminating the error due to the personal characteristics of each observer, the instrument is termed a photoelectric colorimeter. The visible region for the human eye is the wavelength between 400 and 750 nm. The mixture of all colors of different wavelengths in the visible region is known as white light. The visible color is complementary to the color absorbed, that is, it is the color sensation produced by all of the wavelengths minus the wavelengths absorbed. A complete specification of color requires measurements of three recognizable attributes of color (hue, chroma and lightness) (Askar and Treptow, 1993).

Determination

Changing of color during fruit storage was measured in the pulp and peel of mango fruit by Hunter Lab MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Inc., Virginia, USA), which measured color base on the amount of light reflection from the surface. The L*, a* and b* values were measured, and then reported in L* value, chroma or C* ($C^* = a^2 + b^2)^{1/2}$) and hue angle or $^{\circ}H$ ($H = \tan^{-1} b/a$) (McGuire, 1992).

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2. Chemical component changes

2.1 Chlorophyll content of peel

Principle

Chlorophyll is one of the main natural colors in plant. The determination of chlorophyll in fruit is possibly a measure of their stage of maturity. The chlorophyll pigments were extracted in a homogenizer where grinding and extraction occur simultaneously. Solvents like actone and methanol are used which break the chlorophyll-protein complex non-covalent linkage and extract the pigments quantitatively. Undiluted solvents are also block the chlrophyllase activity. To prevent pheophytin formation the extract is generally neutralized with alkaline carbonates. For fruit tris-buffer [(tris-hydroxymethyl0 amino-methane] gives better results. To obtain a clear solution the extracts are filtered by suction through a sintered glass filter or a layer of filter aid. In these extracts chlorophyll can be determined using various methods based on spectrophotometry or spectrofluorometry (Gross, 1987)

Determination

One gram of mango chopped-peel was taken into Erlenmeyer flask. Added 25 ml methyl sulfoxide to the flask, then incubated for 90 minutes at 65°C hot-air oven. After that, it was filtered under suction through sintered glass into suction flask. The filtrate was measured the absorbance at 645 and 663 nm by spectrophotometer (Spectronic 20 D^{+}). The values were calculated:

Chlorophyll a (mg/g FW) = 2.7 (OD 663) – [2.69 (OD 645) x V/ (1000W)]

Chlorophyll b (mg/g FW) = 22.9 (OD 645) – [4.68 (OD 663) x V/ (1000W)]

Toal chlorophyll (mg/g FW) = 20.2 (OD 645) + [8.02 (OD 663) x V/ (1000W)]

Whereas V = volume of analyze solution

W = weight of the peel mango used

OD = absorbance values which read from spectrophotometer

(Witham *et al*., 1986)

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2.2 β-carotene content of pulp

Principle

The determination of caroteniods in fruit is a measure of the stage of maturity and of the quality. β-carotene were extracted using actone-hexane as solvent, and separated from other pigments on magnesium oxide (MgO)/Hyflo Super-Cel (diatomaceous earth) adsorption column, and measured at 436 nm (Greenfield *et al*., 1993).

Determination

Twenty-five grams of mango ground-pulp was taken into a beaker, added 40 ml acetone and 60 ml hexane and then covered with aluminum foil. After standing for 1.5 hours, it was filtered under suction through Whatman No.1 filter paper into suction flask. The residue was added by 2 x 20 ml acetone, then 20 ml hexane. After standing for 30 minutes, it was filtered and washed the residue with 20 ml water, to remove acetone. The extracts were combined and transferred to separating funnel. Five gentle shakes, the extract solution was standing for separating. The lower extract was drained off while the upper extract was transferred into 100 ml volumetric flask, and then adjusted with 9% acetone in hexane. Column was prepared by placing a plug of glass wool into the column, and packed 10 cm of the MgO/diatomaceous earth (non-wash) mixture onto the plug under suction, then added a 1 cm layer of Na_2SO_4. The column was wetted with 50 ml 9% acetone in hexane and applied 50 ml of the extract into the column, washing all the extract onto the column with about 50 ml 9% acetone in hexane. The desired fraction was evaporated on a rotary evaporator and adjusted to a known volume with 9% acetone in hexane (usually 100 ml) in a volumetric flask. The absorbance was measured at 436 nm. β-carotene was calculated and recorded in mg percent (mg%) (Hodge and Hofreiter, 1962).

2.3 Total soluble solids (TSS)

Principle

Soluble solids of the fruit contain sugar soluble solids and non-sugar soluble solids (acids). A concentration of soluble solids in a large volume of fruit juice may be determined by hydrometry or refractometry. Refractometry is an appropriate method if only small samples are available. Hydrometers are hollow glass “spindels” terminating at the lower end in a weighted bulb and having the upper end in the form of a slender stem within which a graduated scale is sealed. When floated in a juice, a hydrometer sinks to a depth determined by the specific gravity

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of the juice, which is related to the concentration of sugar and other soluble solids. Brix (or Balling) hydrometers are calibrated to read directly the percentage by weight of sucrose in pure solutions of sucrose in water. The measurement of soluble solids using a refractometer is the most usual test for routine control purposes. The instrument generally used for determining TSS in fruit processings is either an Abbe or hand refractometer. Although refractometers differ in design, all use the critical angle of total reflection to measure refractive index. The observer sees an optical field partly obscured by a shadow with a sharp boundary, the position of which is determined by the refractive index of the sample (Askar and Treptow, 1993).

Determination

TSS was determined using ATAGO hand refractometer (0-32%). The value was recorded as percent (%).

2.4 Glucose, Fructose and Sucrose Contents

Principle

Occasionally, it is necessary to separate, identify and determine quantitatively the individual sugars in a fruit. Individual sugars can be separated and detected quantitatively by high-performance liquid chromatoghaphy (HPLC). The advantage of HPLC of trimethylsilylether of sugars included faster preparation, ability to chromatogram aqueous solutions, and the direct, non-derivative analytical route. Furthermore, HPLC-chromatograms are more easily interpreted since each sugar yields only one peak (Askar and Treptow, 1993).

Determination

Ten grams of mango tissue were homogenized with 40 ml 85% ethanol at 2°C, and the pH was adjusted to 7.0±0.5 with 0.5 N NaOH to increase the pH. The sample was incubated at 50°C in water bath for 30 minutes. The solution was immediately filtered through a Whatman No. 541 filter paper into a 250 ml flat-bottom, short-neck flask. The extraction was repeated with 25 ml 85% ethanol for three times. The ethanol was evaporated on a rotary evaporator at 50°C, leaving behind an aqueous solution of approximately 3 ml. The aqueous solution was transferred using a syringe to a 50 ml volumetric flask and made up to volume with distilled water. The solution was passed through a 0.45 μm ultrafilter as an analytical sample. Injected 10 μl analytical sample into Shimadzu HPLC (SHIMADZU Co., Kyoto, Japan), which had the condition used 6.5 x 300 mm Sugar Pak I column and All-guard ™ Guard cartridge at 70°C,

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helium gas was the carrier gas in mobile phase (deionized water) with a refractive index detector (RID-6A). A standard solution of sugars was also injected to check retention times and calibration of peak areas. Weight of sugar was calculated and recorded in g/100g sample.

$$\frac{\text{Area sugar} \times \text{Vol. Std. (µl)} \times \text{Conc. Std. (g/100ml)} \times 100}{\text{Area Std.} \times \text{Vol. Sample (µl)} \times \text{Wt. Sample (g)} \times 1}$$

Whereas
Area sugar = values of sample which read from HPLC
Area Std. = values of standard sugar which read from HPLC
Conc. Std. = concentration of standard sugar which used
Vol. Std. = volume of standard sugar which used
Vol. Sample = volume of sample which used
Wt. Sample = weight of sample which used (Bartolome *et al*., 1996)

2.5 pH value

Principle

The term pH is the symbol for hydrogen-ion concentration, and is defined as the logarithm of the reciprocal of hydrogen ion concentration in g/liter.The pH is measured directly with a pH meter which indicates the effective acidity of the juice (Greenfield *et al*., 1993).

Determination

The pH of mango juice was measured by pH meter Orion 420A.

2.6 Titratable acid content

Principle

Titratable acidity (TA) deals with measurement of the total acid concentration contained within a food. TA can be measured by titration with standard alkali to an end-point depending on the selected indicator, such as to a faint pink color with phenolphthalein indicator or to a pH of 8.1 with a glass electrode pH meter (Ranganna, 1986), express in terms of the predominated acid (Sadler and Murphy, 1998).

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Determination

Two ml of mango juice were titrated with standard sodium hydroxide (0.1 N NaOH) solution using pH meter at pH 8.1. Titratable acid was expressed in terms of g of citric acid/100 ml juice extract (AOAC, 2002).

2.7 Citric acid and malic acid contents

Principle

Organic acids can be separated and detected quantitatively by HPLC, which is often the technique of choice for organic acid determination in fruit and fruit products (Askar and Treptow, 1993).

Determination

Ten grams of mango tissue were homogenized with 40 ml 85% ethanol at 2°C, and measured the pH. Add sufficient 0.5 N NaOH to increase the pH to 7.0±0.5. The sample was placed on 50°C water bath for 30 minutes. The solution was immediately filtered through a Whatman No. 541 filter paper into a 250 ml flat-bottom, short-neck flask. The extraction was repeated with 25 ml 85% ethanol for three times. The ethanol was evaporated on a rotary evaporator at 50°C, leaving behind an aqueous solution of approximately 3 ml. The aqueous solution was transferred by a syringe to a 50 ml volumetric flask and made up to volume with distilled water. The solution was passed through a 0.45 μm ultrafilter as an analytical sample. The analytical sample was injected 20 μl into Shimadzu HPLC (SHIMADZU Co., Kyoto, Japan), which had the condition used 8 mm ID x 300 mm Rspak KC-811 column and Rspak KC-G Guard cartridge at 40°C, helium gas was the carrier gas in mobile phase (0.1% phosphoric acid) with a refractive index detector (RID-6A). A standard solution of acids was also injected to check retention times and calibration of peak areas. Weight of acid was calculated and recorded in g/100g fresh weight (Bartolome *et al*., 1996).

2.8 Vitamin C content

Principle

The ascorbic acid (vitamin C) content can be estimated by macerating the sample with a stabilizing agent, metaphosphoric acid to maintain proper acidity for reaction and to avoid autooxidation of ascorbic acid at high pH and reduction of 2,6-dichlorophenol indophenol (this dye is blue in alkaline solution and red in acid solution). 2,6-dichlorophenol indophenol can be

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reduced by ascorbic acid in solutions to a colorless form. The reaction is practicall specific for ascorbic acid in solution in the pH range 1-3.5 (Askar and Treptow, 1993, Greenfield *et al*., 1993).

Determination

Ten ml of mango juice were added by 25 ml of 3% metaphosphoric acid solution, and then titrated with 0.005% indophenol dye, to pink persisting 15 seconds. Two ml of standard ascorbic acid solution were added with 5 ml of acid solution, and then titrated with dye for standardization of dye. Vitamin C was calculated and expressed in terms of mg ascorbic acid/100 ml juice (AOAC, 2002).

2.9 Starch content

Principle

Most immature and mature fruits contain starch. The starch accumulates during maturity of fruit and rapidly lost during ripening. The loss is evident in the chloroplast where the starch granules become progressively smaller as ripening proceeds. Starch granules completely disappear in the ripe fruit. The decreasing of starch indicates ripening of fruit. The hydrolysis of starch and formation of sugars have been associated with amylase activity and can be measured using amount of glucose (Askar and Treptow, 1993).

Determination

Ten grams of blended mango tissue were added with 100 ml of distilled water, 5 ml of 12% zinc acetate and 5 ml of 6% potassium ferrocyanide, then stood for 15 minutes. The solution was filtered through a Whatman No. 4 filter paper into a 250 ml volumetric flask. The extraction was repeated with 25 ml mixture of 1 ml of 12% zinc acetate and 1 ml of 6% potassium ferrocyanide in 200 ml water. The solution was stood for 10 minutes, and filtered through the same filter paper. The residue was rinsed on the paper with 40 + 50 ml of 1.5 N HCl into 250 ml Erlenmeyer flask. The sample was placed on 90°C water bath for 1.5 hours. After that, it was removed from the water bath and immediately cooled. It was adjusted to alkalinity with 20% NaOH (about 27 ml), added 10 ml of HCl solution (HCl: water = 1: 2). The Erlenmeyer flask was rinsed with 15 ml of 20% phosphotungstic acid and 15 ml water to volumetric flask, and adjusted volume. It was stirred and stood for 30 minutes and then filtered the solution through a Whatman No 1 filter paper. Reducing sugar was determined by dinitro salicylic acid (DNS)

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method. Starch content was calculated and recorded in percent fresh weight (AOAC, 2002, Khalafalla and Palzkill, 1990)

2.10 Dry matter content

Principle

Dry matter of plant is its solids substants such as carbohydrate, protein, fat and minerals, excluding water. It is a measurement of the mass of these substant when completely dried. Dry matter can be measured using some tools for drying such as a hot air oven and an "Infared Moisture Balance" which provides continual indication of weight decrease or moisture loss and dry weight or dry matter. The Infared Moisture Balance is especially useful since weighing and drying are simultaneous. Some produces such as potato, the dry weight is estimated by determining the specific gravity and using the equation for calculating (Askar and Treptow, 1993).

Determination

One gram of mango homogenated tissue was placed in Sartorius MA 30 moisture balance (Sartorius AG Gottingen Co., Germany). The dry matter was measured and recorded as percent.

2.11 Water-soluble pectin content

Principle

Increased solubility of pectic polysaccharides is one of the most universal features of ripening fleshy fruit. The mechanisms contributing to this process have not been fully elucidated, though the magnitude of solubility increases varies greatly among different fruit. During ripening, the fruit cell walls are degraded, releasing the combined monosaccharides of the pectin complex, and the water-soluble pectic materials in the cell walls, losing arabinose and galactose accounting for the galacturonan-rich polysaccharides in the mesocarp (Huber, 1983).

Determination

Pectin extraction

Five grams of mango pulp were homogenized with 40 ml of 95% ethanol at 85°C in blender. The homogenate was heated in a water bath at 85°C for 10 minutes, and centrifuged at 10,000 x g for 15 minutes. The pallet was rinsed with 65% ethanol. Twenty-five ml of the 65% ethanol solution were added and the sample was heated at 85°C for 10 minutes. The sample was

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centrifuged at 10,000 x g for 15 minutes. Then, the pallet was rinsed with water, re-suspended in 30 ml of water and centrifuged at 10,000 x g for 15 minutes. Twenty-five ml of 0.1 N NaOH were added into the supernatant and the volume was adjusted to 50 ml with distilled water. Finally, the supernatant was determined for water-soluble pectin (WSP).

Pectin determination

The 0.1 ml supernatant was diluted to 10 ml with distilled water and then placed in ice-water bath. Next, 5 ml of 0.0125 M sodium tetraborate solution (in the concentrated H_2SO_4) were added. After that, the sample was heated in a water bath at 100°C for 10 minutes, cooled down in ice-water immediately, and then the 0.2 ml of 0.4% carbazole was added and repeated to heat and cooled again. The absorbance was measured at 530 nm. The standard curve was prepared based on galacturonic acid as standard. Water soluble pectin content was calculated and recorded in g D-galacturonic acid/100g alcohol-insoluble solids (AIS) (Miller *et al*., 1987, Naohara and Manabe, 1994).

3. Physiological changes

3.1 Respiration and ethylene production rates

Principle

Respiratory behavior and ethylene production are the two of most important indices of physiological activities of fruit and vegetables. Respiratory metabolism recaptures stored energy released from carbohydrates and other energetically rich organic compounds and generates carbon skeletons for reactions needed for the maintenance and development of the harvested fruit. The rates of these processes can be reduced by restricting the availability of O_2, which is a substrate in the terminal step of respiratory pathway, and, to a more limited extent, by elevating the concentration of the product of respiration, CO_2. Restricting the rate of respiration results inshifts in primary metabolism that have the potential to improve or impair quality (Mir and Beaudry, 2002). Ethylene is a simple gaseous hydrocarbon that can diffuse into and out of plant tissues, from both endogenous and exogenous sources, and it can profoundly affect quality factors of horticultural produce such as color, texture and flavor. These effects can be beneficial or deleterious depending on the produces and its uses. The determination of respiration rate and ethylene production can be measured by gas chromatography (Watkins, 2002).

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Determination

These were measured by gas chromatography. Four replicated fruits for each treatment (weighing about 1.5 kg) were allowed to respire in 1.5 L glass jar. One milliliter of head space gas was collected, using a gas-tight plastic syringe and injected into Shimadzu GC-8A gas chromatography for CO_2 analysis and Shimadzu GC-14A (SHIMADZU Co., Japan) for C_2H_4 analysis. The condition of Shimadzu GC-8A used PorapackQ column at 50°C, helium gas was the carrier gas with a thermal conductivity detector. For Shimadzu GC-14A, PorapackQ column was used at 85°C, nitrogen gas was the carrier gas with a flame ionization detector. Respiration rate and ethylene production were recorded as mg CO_2/kg.h^{-1} and µl C_2H_4/kg.h^{-1}, respectively.

4. Enzyme activity changes

Principle

Both pectinmethylesterase (PME) and polygalacturonase (PG) are found in the fruit. PME de-esterifies the methyl group of pectin, converting it into low methoxy pectin or pectic acids, whereas PG hydrolyses the glycosidic linkages in pectic substance, decreasing viscosity significantly. The activities of these enzymes result in softening of plant tissue and cause cloud separation and viscosity loss in fruit. PME activity can be determined titrimetrically by estimating the free carboxyl groups formed in pectin as a result of enzyme action. Activity of PG can be followed by measuring the increase of reducing groups due to the enzymatic action on the glycosidic bonds (Le Roux, 1996).

4.1 PG activity

Determination

Enzyme extraction

Twenty-five grams of mango pulp were homogenized in 25 ml of cold distilled water in the blender. Then, the suspension was centrifuged at 10,000x g for 15 minutes. The precipitated pallets were re-suspended with 25 ml of 0.2 M Tris-HCl buffer (pH 9.0) that contained 5% NaCl. The 1% (W/V) polyvinyl-polypyrrolidone (PVP-40) was added to the suspension. The mixture was stirred for 2 hours, centrifuged at 15,000x g for 20 minutes, and added by ammonium sulfate

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until saturation. Protein was collected by centrifugation. The pallet was dissolved with small amount of water, and dialyzed with 20 mM sodium acetate buffer (pH 6.0). All steps were done at 4°C.

Enzyme activity

PG activity was determined by measuring the formation of reducing groups in a reaction mixture at 37°C, which initially contained 0.25 ml of 0.5% polygalacturonic acid, 0.25 ml of 0.4 M NaCl, 0.25 ml of 0.4 M sodium acetate buffer (pH 4.5) and 0.25 ml of enzyme solution. The reaction was terminated by adding 0.5 ml of 30% K_2CO_3 solution that contained 5% $Na_2S_2O_4$. The reaction mixture was added with 0.5 ml of 0.3% 3,6-dinitrophthalic acid monopyridium salt, and boiled for 10 minutes. After cooling to room temperature, the absorbance was measured at 450 nm. Protein concentration was determined by Bradford method (Yoshida *et al*., 1984)

4.2 PME activity

Determination

Enzyme extraction

Twenty-five grams of mango pulp were homogenized in 25 ml of cold distilled water in the blender. Then, the suspension was centrifuged at 10,000x g for 20 minutes. The precipitated pallets were re-suspended with 25 ml of 1 M NaCl, and 1% (W/V) polyvinyl-polypyrrolidone (PVP-40) was added to the suspension. The mixture was stirred for 2 hours, centrifuged at 15,000x g for 20 minutes. The supernatant was adjusted to pH 7.5 with 2 N NaOH, and PME activity was determined. All steps were done at 4°C.

Enzyme activity

PME activity was determined by 2 ml of pectin substrate solution (pH 7.5) that contained 0.5% (w/v) pectin (Sigma), 0.2 ml of 0.01% bromthymol blue in 3 mM potassium phosphate buffer, 0.7 ml at 25°C. The reaction started when 100 µl of the crude extract was added, then measured the absorbance at 620 nm by distilled water as a blank. Protein concentration was determined by Bradford method (Hagerman and Austin, 1986, Miller *et al*., 1987)

Protein assay (Bradford method)

Dye stock solution

Coomassie Blue G (100 mg) was dissolved in 50 ml methanol. The solution was added with 100 ml of 85% phosphoric acid (H_3PO_4), and added with distilled water up to 200 ml of

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water. The solution appeared dark red in color. The final reagent concentrations were 0.5% mg/ml Coomassie Blue G, 25% methanol and 42.5% H_3PO_4. The solution was stabled indefinitely in a dark bottle at 4°C.

Working solution

The assay reagent was prepared by diluting 1-fold of dye stock solution with 4-fold of distilled water. The solution appeared brown in color, with a pH of 1.1. It was stabled for few weeks in a dark bottle at 4°C.

Standard protein

Standard protein was prepared in the same buffer as the samples to be assayed. A standard protein was bovine serum albumin (BSA) with concentrations of 0 – 0.1 mg/ml. The standard curve plotted the graph and used for protein assay.

Protein assay

One ml of crude extract was mixed with 4 ml of assay reagent and incubated for 30 minutes. Then, it was analyzed in spectrophotometer (UV-Visible spectrophotometer, Shimadzu) at absorbance of 595 nm. The obtained results were recorded and compared with standard protein (Bradford, 1976).

All chemicals used in this study were the laboratory grade chemical from Merck & Co. Inc., Germany and Sigam-Aldrich, Inc., USA.

5. Sensory evaluation

Principle

Sensory evaluation of fresh fruits provides a practical and rapid test for quality. Sensory analysis determines mainly the following quality attributes: appearance (including color, size, shape, uniformity and absence of defects) and taste and flavor (including texture, consistency, viscosity, feeling, taste and odour). Quantitative difference tests are used for determining order acoording to one or more specific characteristic or determining preference used in product and process improvement, selection of the best sample, consumer preference analysis and also the selection of trained panel. The common quantitative difference tests used are ranking, numerical scoring (used in this study) and composite scoring tests (Askar and Treptow, 1993, Sauvageot, 1996).

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Determination

The sensory evaluation included texture, feeling, consistency and taste were carried out with 20 volunteers (10 men + 10 women), recruited from the Faculty of Engineering and Agro-Industry at Maejo University. Panelists were trained on descriptive analysis, using the quantitative difference tests. An intensity scale is a numerical scoring from 1 to 5, with being: 5 = excellent or perfect, 4 = good, 3 = fair, 2 = poor and 1 = off or unacceptable (Askar and Treptow, 1993).

Experiment 2 Changes on quality and enzyme activity during ripening of 'Keaw Morakot' mango fruit at low temperature

'Keaw Morakot' mango fruits were harvested from a local commercial orchard in Baan Hong district, Lamphun province. The fruit were transported to the laboratory of the Department of Postharvest Technology, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai. Upon arrival, the fruit were washed in 100 ppm sodium hypochlorite for 5 minutes. After washing, the fruit were floated in water for using in this experiment. The fruit which floated in water were discarded. After that, the fruit which sank into water were sorted into 3 maturity levels, by floating in 3 concentrations of brine solution, 4, 6 and 8% (specific gravity 1.04, 1.06 and 1.08, respectively), as Maturity 1, Maturity 2 and Maturity 3. The fruit were stored at 13°C, 85-90% RH for 21 days. During storage at low temperature, the mango fruit were taken to measure the qualities every 3 days. After storage for 21 days, the fruit were ripened at 25°C, 70-75% RH for 7 days and measured the qualities everyday. The sensory was tested only on day 7.

The experiment was arranged in a completely randomized design (CRD) with three treatments. All treatments were replicated three times. The data were analyzed by performing analysis of variance, using SX-statistic program. Mean comparison was done by the least significant difference test (LSD) at $\rho = 0.05$.

The parameters were examined the same as in the Experiment 1. For sensory evaluation in the Experiment 2, off-odor was also tested. An intensity scale was a numerical scoring from 1 to 5, with being: 5 = extremely high, 4 = high, 3 = moderate, 2 = slightly and 1 =none.

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Experiment 3 Effects of 1-MCP on the changes of quality and enzyme activity during ripening of 'Keaw Morakot' mango fruit at low temperature

'Keaw Morakot' mango fruit were harvested from a local commercial orchard in Baan Hong district, Lamphun province. The fruit were transported to the laboratory of Department of Postharvest Technology, Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai. Upon arrival, the fruit were washed in 100 ppm sodium hypochlorite for 5 minutes. The fruit were sorted at maturity levels with the best quality from Experiments 1 and 2, by floating in brine solution 6% (specific gravity 1.06). The mango fruit were placed in 0.25 m^3 polyethylene chamber, together with a beaker containing the amount of EthylBloc® powder (Rohm and Hass, Ltd.) needed to generate the required volume of 1-MCP. Water was added to the beaker to release the 1-MCP gas and the chamber was immediately closed for the desired exposure period. A control unit was treated in the same manner, but without the addition of EthylBloc® powder. The concentrations of 1-MCP used were 0, 500 and 1,000 nl/l 1-MCP, and the exposure times were 6 and 12 hours at 20°C. After treating with 1-MCP, the fruit were stored at 13°C, 85-90% RH, and then measured every 3 days for 21 days.

The experimental design was arranged in a 3 x 2 split-plot in CRD, concentrations = main plot, exposure times = sub plot). All treatments were replicated three times. Results were analyzed by performing analysis of variance, using SX-statistic program. Mean comparison was done by the least significant difference test (LSD) at $\rho = 0.05$.

The parameters were examined same as the Experiment but no sensory evaluation in the Experiment 3.

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