การใช้ฉายรังสีร่วมกับการใช้ความร้อนในน้ำมะม่วงเข้มข้น

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บทคัดย่อ

การศึกษาผลของการฉายรังสีรวมกับการใช้ความร้อนเพื่อยืดอายุการเก็บรักษาและคุณภาพของน้ำมะม่วงเข้มข้น (Mamgo puree) การยับยั้งปฏิกริยาของเอนไซน์ โพลีฟีนอล ออกซิเดซ (Polyphenol Oxidase) โดยการใช้ความร้อนที่อุณหภูมิ 80 °C นาน 15 นาทีหลังจากการใช้ความร้อนแล้วน้ำมะม่วงเข้มข้นถูกฉายรังสีที่ 0 , 2 , 4 , 6 และ 8 กิโลเกรย์ และผลของความชื้น, pH, ปริมาณกรด, และปริมาณน้ำตาลของน้ำมะม่วงเข้มข้นปรากฏว่าไม่มีการเปลี่ยนแปลงในช่วงการเก็บรักษาในน้ำมะม่วงเข้มข้นที่ฉายรังสีในระดับต่ำจะมีผลทำให้เกิดการเจริญเติบโตของ จุลินทรีย์ และเกิดการเปลี่ยนแปลงคุณภาพของน้ำมะม่วงเข้มข้น การฉายรังสีรวมกับการใช้อุณหภูมิต่ำ (5 °C) สามารถลดอัตราการเปลี่ยนแปลงเป็นสีน้ำตาลของน้ำมะม่วงระหว่างการเก็บรักษา ผลของการฉายรังสีที่ 0 ถึง 8 กิโลเกรย์ จะทำให้ปริมาณของจุลินทรีย์ ลดลงเป็นไปตามสมการเอ็กซ์โพเนนเชียล แต่ที่ 6 และ 8 กิโลเกรย์ ปรากฏว่าไม่มีการเจริญเติบโตของ จุลินทรีย์ และที่ 8 กิโลเกรย์ จุลินทรีย์ ไม่สามารถเจริญเติบโตได้เมื่อเก็บรักษาทั้งที่อุณหภูมิบรรยากาศและที่อุณหภูมิ 5 °C

Irradiation in Combination of Heat Treatment of Mango Puree

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ABSTRACT

The effect of irradiation with heat combination treatment on the shelf life and quality of mango puree was studied. Thermal inactivation of polyphenol oxidase enzyme at 80°C and 15 min was used as a measure of adequacy of pre-heat treatment. Irradiation of mango puree after heat treatment at dosage of 0, 2, 4, 6 and 8 kGy showed no change in mc, pH, acidity, and TSS but during storage, growth of microorganisms brought changes in these values.

Irradiation in combination with low temperature (5°C) reduced discoloration and darkening rate during storage. Irradiation dose from 0 to 8 kGy resulted in log linear reductions in microorganism levels but at 6 and 8 kGy, there was no growth of microorganisms. Products irradiated at 8 kGy showed no microorganism growth at both temperatures.

INTRODUCTION

The leading mango cultivars in Thailand are Okrong, Rad, Namdokmai, Nang Klangwan, Tongdum, and Kaew (Vangnai et al., 1984). Mangoes are processed into puree for remanufacturing into finished products such as nectar, juice, squash, jams, jellies and dehydrated products. Preservation of puree facilitates availability of mango, though in a derived form, throughout the year, which could be used for processing into other products. The storage of puree is economical compared to the cost of storing the fresh mangoes, except those that are dehydrated.

Low level gamma irradiation has proved to be an effective technique to extend the shelf life of tropical fruits such as mango, banana and papaya. But not much research has been undertaken on irradiation of mango puree. Irradiation treatment is not very effective on liquid products but mango puree is considered as thick liquid (17-20 °Brix) and could be treated by irradiation. Lodge et al. (1985) irradiated frozen (-18 °C) de-seeded Kiwifruit pulp (*Actinida deliciosa*) at a dose of 1 kGy and stored at -18 °C. Irradiation resulted in a 2.11 $\log_{10}$ reduction in aerobic plate count (APC). No significant differences in physical, chemical and sensorial properties between irradiated and nonirradiated pulps were observed over 6 months of storage. They further reported that three days frozen storage (-18 °C) following irradiation resulted in an APC of only 0.89 $\log_{10}$ higher than 6 months frozen storage without irradiation. Some reports about the effects of gamma radiation and refrigeration of guava puree concentrate showed that nonirradiated canned guava puree spoiled after 2 days at 24 °C and 4 to 6 days at 7.2 °C. The storage life of guava puree irradiated at 1 kGy was extended 4 to 6 days when stored at 24 °C, and still longer when stored at 7.2 °C. Irradiation with 5 and 10 kGy increased the cell counts in samples stored at 24 °C, but decreased with storage at 7.2 °C.

The problem is not only of shelf life. Browning reaction or discoloration of mango during processing occurs seriously when the tissue is injured, peeled or diseased. This reaction is due to the conversion of phenolic compounds to quinones by polyphenol oxidase (E.C.1.10.3.1), then undergoing polymerization to impact brown discoloration. Park et al. (1980) extracted polyphenol oxidase (PPO) from mango (*Mangifera indica* var. Harden) and investigated in order to use it in mango processing. The optimum pH of the enzyme was 5.6-6.0. For heat inactivation, half-activity of the enzyme was lost after 2.1 and 4.0 min of treatment at 85 and 80 °C, respectively.

Nair et al. (1972) studied the effect of polyphenol oxidase activity on irradiated mango and found considerable increase in polyphenol oxidase activity with an increase of irradiation dose from 1 to 2 kGy. Thomas and Janave (1975) concluded that irradiation injuries of skin and pulp increased with increasing doses and prolonged storage period due to increased polyphenol oxidase activity.

Pre-heat treatment should be done before irradiation to inactivate polyphenol oxidase due to considerable increase in its activity with an increase of irradiation dose. However, discoloration of mango puree may not occur from only enzymatic browning reaction, as nonenzymatic browning mechanism can cause darkening of the product during storage.

Therefore, this study aimed at investigating the possibility of using combined irradiation and heat treatment in prolonging the market life of mango puree. Thermal

inactivation of polyphenol oxidase, quality attributes, discoloration and microbiological level were investigated and compared during storage at ambient and refrigerated temperatures.

MATERIALS AND METHODS

Mango Puree Preparation

Unripe, matured mangoes (*Magnifera indica L.* var. Kaew) were ripened with calcium carbide. Ripe mangoes were washed, sliced into pieces with a stainless steel knife and seed was removed. Pieces of mangoes were put into pulp-finisher machine. Puree samples were packed in 16X150 mm test tubes.

Thermal Inactivation of Polyphenol Oxidase Enzyme in Mango

Thermal inactivation of polyphenol oxidase enzyme was investigated and used as the measure of the adequacy of pre-heat treatment. Mango puree was put in 16X150 mm test tubes and heated in a water bath at 60, 70, 80 and 90 °C. After various heating intervals, polyphenol oxidase activity in the extract was detected spectrophotometrically at 25 °C with a visible spectrophotometer (UNICAM 8620 UV/VIS, Japan). The absorbance at 470 nm was recorded at every 2 min interval for 10 min. Blanks were prepared without the addition of guaiacol and H_2O_2 (Ranganna, 1986).

One unit of activity is defined as a change in absorbance of 0.001 per min (Hemeda & Klein, 1990). Log of activities at various temperatures was plotted against pre-heating times to present the thermal resistance curve of the crude enzyme extract.

Irradiation of Mango Puree

Mango puree samples were heated at 80 °C for 15 min (which was selected from the previous experiment) to inactivate polyphenol oxidase enzyme. Mango puree packed in test tubes were arranged in the racks and were placed inside the irradiation chamber. The samples have been irradiated at minimum requested doses of 0, 2, 4, 6, and 8 kGy at the Office of Atomic Energy for Peace, Bangkok, Thailand, by using Gammacell 220. After irradiation, the samples were analyzed for chemical and microbiological characteristics, and stored at ambient and 5 °C conditions.

Physical and Chemical Analyses

The moisture content was determined by oven drying method at temperature of 50 °C (Ranganna, 1986). Titrable acidity was determined by titrating sample solutions against standard 0.1 N NaOH using a few drops of phenolphthalein as indicator and calculated as % anhydrous citric acid (Ranganna, 1986).

Total soluble solids (TSS) content was recorded as °Brix by using an Abbe refractometer (AOAC, 1984). The pH was measured by immersing the electrode into the puree (AOAC, 1984). The pH meter was calibrated using 4 and 7 pH buffer.

Hunter L, a and b values were determined using a Color and Color Difference Meter (Denshoku, Model TC P-III, Japan) which was standardized against a white plate. Color measurements (as Hunter L, a, and b) were recorded by placing the sample on the top of the optical unit.

Microbiological Method

Viable microorganisms were enumerated by using aerobic plate count (APC) technique. The number of colony forming units (cfu) on each plate were counted and presented as cfu/g of the sample.

RESULTS AND DISCUSSION

Thermal Inactivation of Polyphenol Oxidase Enzyme in Mango Puree

Polyphenol oxidase enzyme was used as an index of adequacy of pre-heating time because of its relatively higher thermal stability over other plant enzymes. Fig. 1 shows the results of heat inactivation of polyphenol oxidase enzyme as % relative activity at 60, 70, 80 and 90 °C. When the logarithm of the relative activities was plotted against time interval of heat treatment, the lines were obtained with slope dependent on temperature as shown in Fig.2. The rate of thermal inactivation was proportional to increasing temperature and enzyme activity lost rapidly at 80 and 90 °C. The observed temperature and time of inactivation were 80°C and 15 min.

Physical and Chemical Analyses

The average moisture content, pH, acidity and TSS of the mango puree were 81.76 %, 4.55, 0.072 % and 17.43 °Brix, respectively. They were heated at 80 °C for 15 min and irradiated at different dosage ranging from 0, 2, 4, 6 and 8 kGy. There was no change in moisture content, pH, acidity and total soluble solids after irradiation at different dosages. The products kept at ambient temperature for 60 days of storage tended to have lower pH and higher acidity than those kept at 5 °C. After 60 days of storage at 5 °C, TSS of puree remained constant, while at ambient temperature, there was slight decrease in 2 kGy samples.

The effect of irradiation on mango puree color value (Hunter L, a, b, and angle) is illustrated in Fig. 3. Statistically, Hunter L and b values did not change with increasing irradiation dose but changed significantly with storage time and temperature (Table 1 & 2). Lightness and yellowness of puree products decreased with storage time. The lightness and yellowness of samples kept at 5 °C were found to be higher than at ambient temperature. Browning reaction directly affected the color of mango puree.

Hunter a and angle ($\tan^{-1}(a/b)$) values changed significantly with irradiation dose, storage time and temperature. Increase in irradiation dosages, storage time and temperature caused more angle value, while Hunter a value increased with irradiation dose but decreased with storage time and temperature.

Discoloration or darkening of mango puree occurred from nonenzymatic browning reaction during storage. Visual observation exhibited that the discoloration occurred from the top of the puree. At the top of test tube, mango puree was exposed directly to the air that remained in the head space of test tube. The results showed that oxidative type of browning reactions could occur.

The appearance of mango puree product is very important, pre-heat treatment can reduce discoloration of the product during processing. But during storage at ambient temperature, color change occurred faster than those that were stored at 5 °C. Thus irradiation in combination with low temperature can preserve this product more effectively than when applied alone.

Microbiological Analyses

Results of microbiological analyses for this experiment are shown in Table 3. The initial microbiological quality of the puree was poor with about 7 X 10^6 cfu/g. Increasing the radiation dose between 0 to 8 kGy resulted in approximately log linear reductions in microorganism levels. APCs were reduced 2.3 log cycles in pre-heat treatment step and 1.02 and 0.26 log cycles at irradiation dose of 2 and 4 kGy, respectively. At 6 and 8 kGy, there was no growth of microorganisms. Most of colony forming units were found to be yeasts. Yeasts could be more radio-resistant than molds.

The growth of microorganisms was observed. Controlled samples that were not irradiated spoiled within 2 days at ambient temperature. In the control samples that were stored at 5°C, APCs increased 0.34 log cycles at 60 days of storage. Sample was fermented within one day after being removed from refrigerator. At 60 days of storage, the irradiated products that were kept at ambient temperature had higher amount of microorganisms than those that were kept at 5 °C. However, at 8 kGy there was no growth of microorganisms for both ambient and refrigerated temperature.

CONCLUSIONS

To reduce discoloration of mango puree during processing, selected temperature and time for pre-heat treatment were 80 °C and 15 min. After irradiation there was no change of pH, acidity, and TSS at different dosages. During storage, changes of these values were occurred at low level of irradiation doses due to the growth of microorganisms in the product. Irradiation with low temperature storage can reduce the change in quality attributes. Pre-heat treatment can reduce discoloration of the product during processing. But during storage at ambient temperature, color change occurred faster than those that stored at 5 °C. Thus irradiation in combination with low temperature can preserve this product more effective than that of alone.

The initial microbiological quality of the puree was about 7 X 10^6 cfu/g. Increasing the radiation dose between 0 to 8 kGy resulted in approximately log linear reductions in microorganism levels. Most of colony forming units were found to be yeasts. At 60 days of storage, the products kept at ambient temperature had higher amount of microorganisms than those that kept at 5°C. At 8 kGy there was no growth of microorganisms for both ambient and refrigerated temperature. Therefore, irradiation with low temperature storage can be promising in prolonging the shelf life of mango puree product.

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Table 1. Effect of irradiation doses, storage period and temperature on Hunter L and a of mango puree.

Irradiation Doses (kGy)	Hunter L Value			Hunter a Value		
	0 day	60 days at ambient	60 days at 5°C	0 day	60 days at ambient	60 days at 5 °C
Control	41.63	*	*	9.71	*	*
0	40.82	*	35.45	9.62	*	8.48
2	41.88	30.64	36.20	9.72	6.95	8.48
4	41.13	31.16	36.46	8.96	8.04	8.84
6	42.26	29.42	36.29	9.92	7.91	9.45
8	41.54	30.73	36.02	9.95	8.24	9.09

Table 2. Effect of irradiation doses, storage period and temperature on Hunter b and Angle values) of mango puree.

Irradiation Doses (kGy)	Hunter b Value			Angle Value		
	0 day	60 days at ambient	60 days at 5 °C	0 day	60 days at ambient	60 days at 5°C
Control	26.31	*	*	20.26	*	*
0	25.28	*	21.01	20.83	*	22.03
2	25.95	17.11	21.86	20.54	22.19	21.22
4	25.42	18.04	21.98	19.43	24.04	21.90
6	26.24	15.69	21.92	20.72	26.77	23.33
8	25.87	17.51	21.37	21.05	25.24	23.03

Table 3. Effect of various irradiation doses, storage period, and temperature on microbiological levels (as log of cfu/g) of mango puree.

Irradiation Dose (kGy)	Aerobic Plate Count (Log cfu/g)		
	0 day	60 days at ambient	60 days at 5 °C
Control	6.84	*	7.18
0	4.54	6.93	4.41
2	3.52	5.74	3.08
4	3.26	4.08	3.02
6	**	2.72	1.53
8	**	**	**

All values in table were averaged from four replicates., * The samples were already spoiled., ** There was no growth of microorganisms.

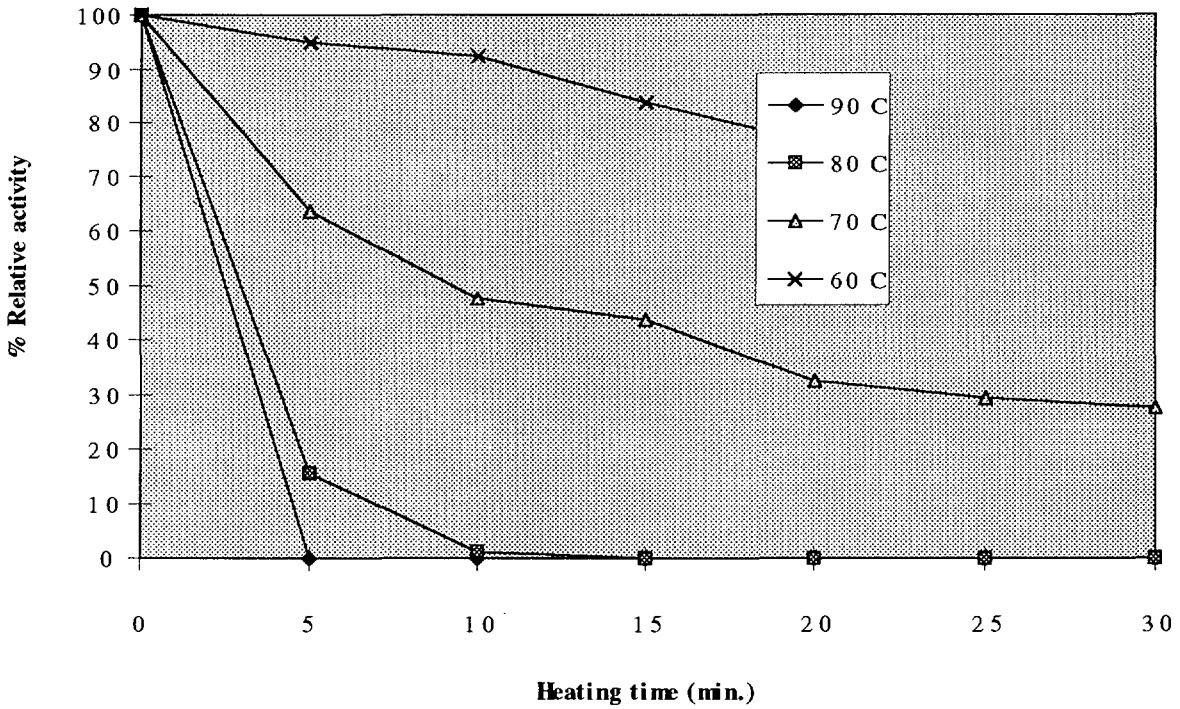

Fig. 1 Heat Inactivation of Polyphenol Oxidase Enzyme as % relative activity at 60, 70, 80 and 90 °C

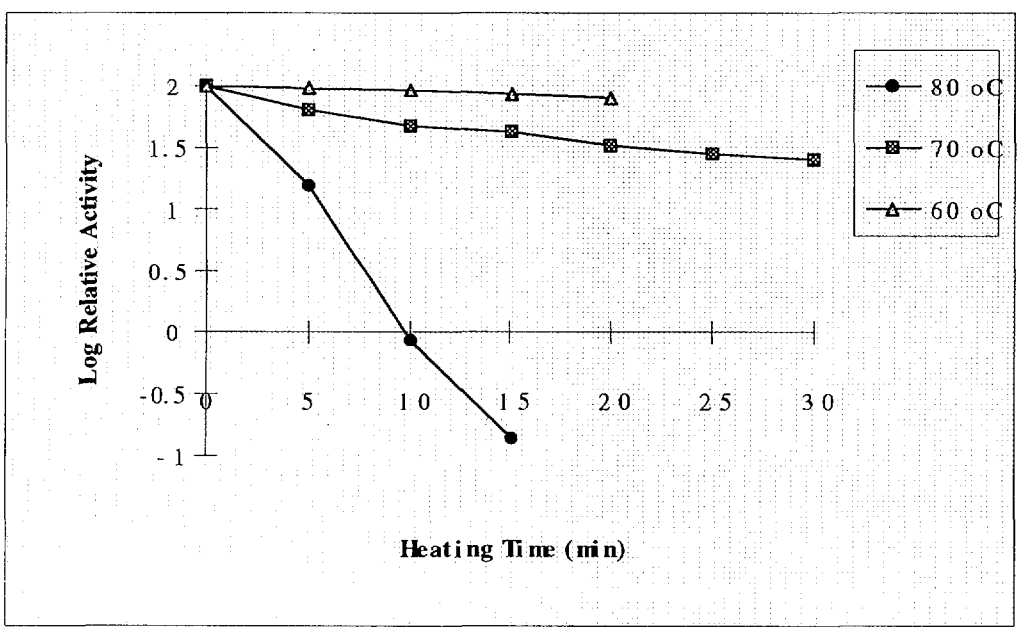

Fig. 2 Heat Inactivation of Polyphenol Oxidase Enzyme as Log of % Relative Activity at 60, 70, 80 and 90 °C.

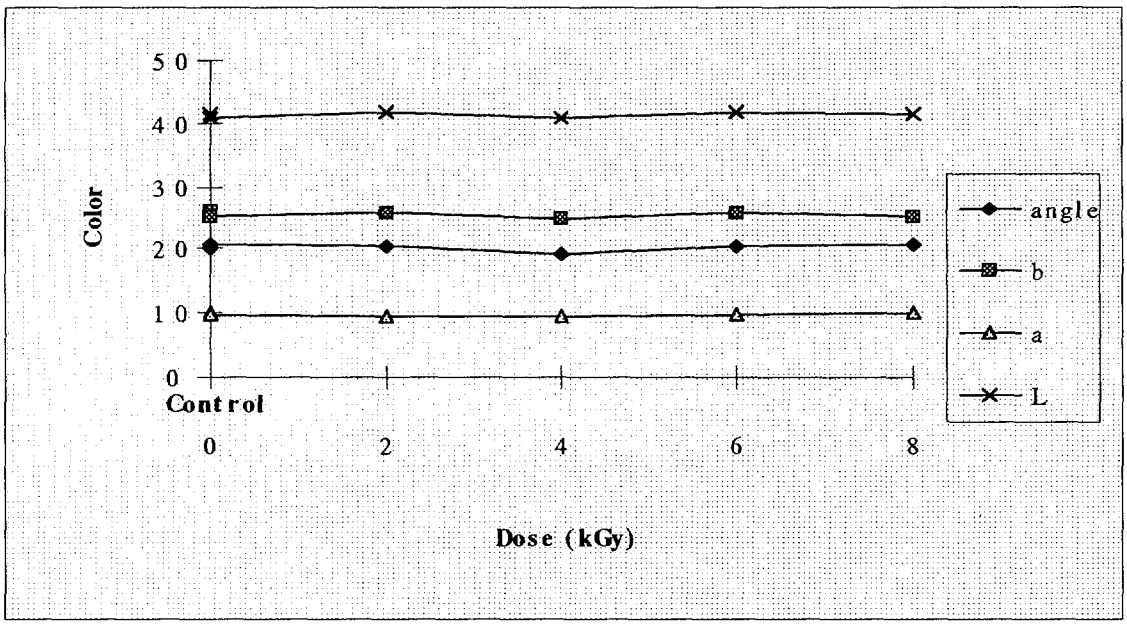

Fig. 3 Effect of Irradiation on Hunter L, a, b and Angle Values of Mango Puree.