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Evaluation of tendering effect from date seed extract (*P. dactalytera*) in knuckle part meat

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Abstract

Phoenic dactylifera (date) is a species of flowering plants in the family of *Arecaceae*. Date seeds are considered as a waste from many processing that produced plants pitted date, confectionery date syrup and date itself. Currently, the seeds are used mainly for animal feed in the cattle and chicken industry. In 2004, about 863,000 tonnes of date seeds are produced out of 6.9 million tonnes date. Tenderness is the major concern that affecting consumer acceptance of beef in meat industry. This study was carried out in order to investigate the effects of the bioactive compound extracted from date seed as a tenderizing agent in meat. Extraction of date seeds used a different method of extraction (Soxhlet and Maceration). The application of extracted on knuckle part of beef were performed and papain was used as a positive control and followed by the sensory evaluation. The analysis of cooked meat was performed in order to analyze the physico-chemical properties of date seeds extract. The result from the study revealed that the aqueous extract (maceration techniques) gave the best percentage of the total yield recovery with 28.44%. The physico-chemical properties of cooked meat showed the reducing of pH value after cooking. Meanwhile for the cooking yield, result showed that almost 86% of water losses during cooking for aqueous extract and positive control and 96% for negative control. According to the sensory evaluation of the cooked meat, scoring test and hedonic test were performed using One Way Anova. The result for texture is 6.10±2.1, juiceness is 5.87±1.76 and taste is 6.80±1.34. All attributes have no significant different at $p < 0.05$ between aqueos extract, and positive control. A general acceptance shows that no significant different between aqueos extract (6.50±2.0) and positive control (7.13±1.98). The result suggested that the tenderization effect of date seed improved the textural properties of knuckle part meat and have potential for tenderization purpose in food industry.

1. INTRODUCTION

Phoenix dactylifera (date or date palm) is a species of flowering plants in the family of oil palm trees, which have an edible and sweet fruits. This species is widely cultivated and is naturalized in many tropical and subtropical regions worldwide. Date seeds are considered waste from many processing industries that produce pitted date, confectionary date syrup and date it. Currently, the seeds are used mainly for animal feed in the cattle and chicken industry. In 2004, about 863,000 tonnes of date seeds are produced out of 6.9 million tonnes date. Several studies have been conducted in the Arab countries that proved date seeds contain nutrients such as carbohydrates, proteins, fats and magnesium (Sumayyah et al, 2012). The use of waste is very important, not only for the cultivation of date but also will increase the income for some sectors. According to Mangino (2007), the physicochemical properties of date seeds are 3.1 to 7.1% for moisture, 2.3 to 6.4% for protein, 5.0 to 13.2% for fat, 0.9 to 1.8% for ash and 22.5 to 80.2% for fibre.

Tenderness is the most important factor that affecting consumer acceptance of beef. Beef tenderness are affected by two primary factors called background tenderness and protein or muscle fiber tenderness (Boles and Pegg, 1999). Background tenderness is determined by the amount and type of connective tissue in any given cut. For example, brisket is generally very tough unless cooked properly, whereas tenderloin is almost always very tender. One major difference between brisket and tenderloin is the amount and type of connective tissue in each. Brisket has more, and tougher connective tissues and tenderloin has less and more tender connective tissues. Protein or muscle fiber tenderness is affected by the strength of the actual meat fibers, which are affected primarily by aging (holding meat in an unfrozen state). Muscle fibers in meat weaken over time due to the action of enzymes, which break apart the fiber, ultimately improving tenderness. Muscle fibers becoming weaker during aging are why steaks aged 14 days are generally tenderer than steaks aged 3 days.

According to Han et al, (2000) meat is basically made of muscles. Each cut of meat is made up of muscle

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fibres bound together by protein filaments called collagen. Tenderizing meat means breaking the long strands of muscle as well as softening the collagen until it turns into gelatine. This soft gelatine will soak into the meat, tenderizing it and adding moisture to make the meat juicy. This tenderizing can be accomplished through physical process like pounding, or through the chemical reactions caused when it is exposed to the acids in marinades and powdered meat tenderizers. The two main ingredients in most powdered tenderizers are papain, found in papayas, and bromelain, found in pineapples. Both enzymes attack the muscle fibres and the collagen webs that hold them together. This will soften the meat and makes it tenderer. This is the reason why raw papaya and pineapples are not incorporated in gelatine-based desserts. The papain and bromelain break down the gelatine, which the same action to collagen in meats (Gerelt et al, 2000). The good nutritional value of date seeds is based on their dietary fibre content, which makes them suitable for the preparation of fibre-based foods. Date seeds are capable to be a source of dietary fiber and significant amount of bioactive phenolics as evaluated by Mangino, (2007).

The aim of this study is to produce a new natural tenderizer from the date seeds through different methods and to investigate the physicho-chemical properties of the extract as well as the quality of tenderizing agent from date seeds extract.

2. MATERIALS AND METHODS

2.1. Plant material

The date seeds (*Phoenix dactylifera)* were purchased from local market in Kuantan Pahang, Malaysia.

2.2. Reagent, chemical and apparatus

n-Hexane, Methanol (MeOH), dichloromethane (DCM) and Ethyl acetate were obtained from Sigma Aldrich Co. St. Louis, USA. Papain were purchased from local supplier. Mill machine (Qingdao Dahua Double Circle), oven dryer (Protech), rotary evaporator (Buchii), electronic balance (Metler Toledo) and Soxhlet apparatus.

2.3. Preparation of crude extracts

The date seeds were washed, cleaned and dried in a dryer at 40°C for two weeks. It was ground, labelled and stored in airtight container for further use.

2.4. Methods of extraction

Extraction were carried out in two different methods; Soxhlet Extraction with different polarity of solvent (n-hexane, ethyl acetate, dichloromethane and methanol) and maceration technique for aqueous part.

2.5. Meat analysis (tenderizing effect on meat)

The knuckle part of beef meat was marinated with distilled water (as a negative control), date seed extracted 1ml/100g, and 2% papain (as a positive control) for 48 hours at 4 ºC. After 48 hours, the sample then were washed and drained. A 2% w/w salt were added and then the sample were cooked using autoclave for 15 minutes at 100°C and 15 psig. Some different parameters have been tested for the meat sample as in Table 1.

Table 1: Evaluated Parameters

No	Evaluated Parameter
A	Date seed (n hexane)
B	Date seed (aquoes)
C	Distilled water
D	Papain (2%)

Only the extract of the aquoues and hexane extracted were selected over others. Papain (2%) as a positif control and Distilled water as a negative control.

2.6. Sensory test

Sensory evaluation was performed comprise of scoring and hedonic test to 30 untrained panelist for the marinated beef. The sensory evaluation was performed in the sensory laboratory with controlled light and temperature. There are three attributes were evaluated which are juiciness, texture and taste.

2.7. Physico-chemical properties (pH, cooking yield, acidity)

The pH level of pre and post cooked sample were determined by using litmus paper. Cooking yield refers to the water loss during cooking process using the autoclave at 100°C, 15 psi for 15 minutes. The titratable acidity was determined using AOAC methods.

3. RESULTS AND DISCUSSION

3.1. Extraction yield

Table 2 shows the percentage of dried sample obtained after the drying process. The percentage of dried sample was 61% of dry weight basis.

Table 2: Percentage of Dried Sample for *P. dactylitera* seed

Sample	Wet weight (g)	Dry weight (g)	% of weight
P. dactylitera seed	1000	610	61%

Table 3 shows the percentage of total yield extractions for different organic solvents and aqueous from both treatments. The total yield of *P. dactylitera* extracts are varied from each solvents and treatments. The highest yields are obtained from aqueous maceration technique and n-hexane solvent (Soxhlet extraction) with 28.44±0.8% and 28.80±0.2% respectively.

3.2. The effect of pH, acidity on treated beef

Table 4 shows the pH values for post-marinating beef meat are within 6 to 7 prior treatments, as there is no enzyme activity occurs during the storage period at 4°C. However, the pH values significantly drop to pH value 4 for papain and 5 for aqueous and methanol treatments. No

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changes in pH values for other treatments. Based on the treatments of 2% papain, 6% methanol and aqueous of *P. dactylitera* extract, it is suggested that pH value has a great influence during the post-autoclaving treatment. As the pH value for that treatments are between 4 and 5, the hardness of the beef is decreased due to the pH value that falls between the isoelectric points of myofibrillar protein. The result indicates that the combination of *P. dactylitera* extract and temperature used was sufficient to weaken and break down the bonds from long range interactions which are necessary for the presence of tertiary structure (Mangino, 2007)

Table 3: Percentage of total extractable yield for *P. dactylitera* seed

Sample	Extraction Yield (%)					Total Extractable Materials (%)
	n-hexane	Dichloro-methane	Ethyl Acetate	Methanol	Aqueous	
P. dactylitera crude extract	28.80±0.2	22.46±0.11	12.79±0.2	10.59±0.5	28.44±0.8	**103.08±1.81**

Yield (%) = (weight of crude extract/ weight of dried sample) x 100%.
Data are mean±standard deviation, n=3

Table 4: Quality of beef meat treated with *P. dactylitera* extract at different temperature

Treatment		Post-Marinating (4°C)			Post-Auctoclaving (100°C)		
		pH	Titrable Acidity (%)g/L as a lactic acid equivalent	Cooking Yield	pH	Titrable Acidity (%)g/L as a lactic acid equivalent	Cooking Yield
P. dactylitera extract 6%	*n*-hexane	6	-	-	6	1.22	84%
	Dichloromethane	6	-	-	6	2.07	89%
	Ethyl Acetate	6	-	-	6	1.78	94%
	Methanol	7	-	-	5	1.66	96%
	Aqueous	6	-	-	5	1.60	86%
Positive control	Papain 2%	7	-	-	4	1.58	86%
Negative Control	Distilled water	7	-	-	7	0.594	96%

(-) = not applicable

3.3. The effect of the volume of cooking yield

Cooking yield refers to the water binding capacity or water holding capacity of the food. Water-holding capacity of fresh meat (the ability to retain inherent water) is important property of the fresh meat as it will affect both yield and the quality of the end product. The result in Table 4, indicates that the result for cooking yield are varies for each treatment. The value for cooking yield for each treatment are: methanol extract (96%), ethyl acetate extract (94%), DCM extract (89%), n-hexane extract (84%) while aqueous and papain extract are 86% respectively. The cooking yield for grilled topside beef was 75%, which slightly lower from this study due to changes in proximate composition, cooking temperature or nutrients become more concentrated during cooking (Habibi 2011). The amount of water that lost during cooking are depending on time, temperature, method of cooking, size of sample, heat penetration and composition leading to an increase in concentration of the fat and protein.

Meat muscle contains approximately 75% of water while the other main components including protein (approximately 20%), lipids or fat (approximately 5%), carbohydrates (approximately 1%) and vitamins and minerals (often analyzed as ash, approximately 1%) (Ashie et, 2002). Meat and meat products are considered cooked when the centre of the product is maintained at a temperature of 65-70°C for 10 minutes since the proteins will then be coagulated and the meat tenderised by partial hydrolysis of the collagen. The vegetative form of bacteria, but not spores, have been destroyed (thermos-resistant spores can survive heating above 100°C). The completion of the cooking process is generally indicated by changes of colour from red to brown (red to pink in cured products) and flavours are developed.

3.4. Sensory evaluation

3.4.1. Scoring test

Table 5 shows the result for scoring test performed using One Way Anova. From the results, all attributes that being evaluated showed that there is no significant different between the treatments using aqueous and papain. The overall result also shows that no significant difference for both aqueous and papain treatments. This indicates that the aqueous extract of *P. dactylitera* has the ability to gives the same effect as per positive control on marinated beef.

Table 5: Scoring test of treated beef meat

Attribute	Negative	Aqueos	Papain
Juiceness	4.30^{a}±2.35	5.47^{b}±1.76	5.93^{b}±1.87
Texture	4.37^{a}±2.61	5.70^{b}±2.20	5.17^{b}±2.1
Taste	5.07^{a}±2.21	4.70^{ab}±1.95	3.90^{b}±2.1
Overall	4.47^{a}±2.16	5.60^{b}±1.98	6.30^{b}±2.17

*different letters in columns are significantly different (P<0.05)

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3.4.2. Hedonic Test

Table 6 shows the result from hedonic test of treated beef meat. The 9-points scale started with the lowest rate for value 1 to the highest rate which represented by value 9 were used. All attributes showed that there is no significant difference between aqueous extract and papain treatments. Overall acceptance also shows there is no significant difference for both treatments. The result indicates that the aqueous extract of *P. dactylitera* have an ability to gives the same effect as papain (positive control) to the marinated beef.

Table 6: Hedonic test of treated beef meat

Attribute	Negative	Aqueos	Papain
Juiciness	$4.23^{a}\pm1.382$	$5.87^{b}\pm1.737$	$7.37^{b}\pm1.712$
Texture	$4.97^{a}\pm1.712$	$6.10^{b}\pm2.057$	$6.80^{b}\pm1.955$
Taste	$4.40^{a}\pm1.163$	$6.80^{b}\pm1.349$	$7.07^{b}\pm1.507$
Overall	$4.17^{a}\pm1.020$	$6.57^{b}\pm1.906$	$7.17^{b}\pm1.984$

*different letters in columns are significantly different ($P<0.05$)

4. CONCLUSION

In conclusion, this study was carried out to extract the crude extract from date seeds using soxhlet extraction and maceration technique. The comparison was made between the solvents for soxhlet extraction and aqueos extract for the maceration. This study revealed, the total extractable yield from date seeds is 103.80% with the best recovery is from aqueos extract (28.44±0.8%) and n-hexane (28.80±0.2%). The physico-chemical properties of each crude extracts from different extraction methods were analyzed. pH value were different between post-marinating and post-autoclaving. The result shows that papain able to reduce the pH from 7 to 4 during post-autoclaving compared to pH value of 5 for aqueos extract. Distilled water (negative control) shows the highest value for cooking yield with 97% compared to 86% for both papain (positive control) and aqueous extract. Sensory evalution showed that there is no significant difference between aqueous extract with papain treatments. This result showed that *P. dactylitera* extract have a potential use as a meat tenderizer.

Future directions of this project are proposed in line with current commercial meat tenderizer. The direction includes to study the effect of extract toward meat texture. The rational of the study is to provide a great choice in the market for tenderizer. Finally, the study also will identify the bioactive compound that responsible for tenderizing effect on the meat.

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