RETARDATION OF LIPID OXIDATION IN PORK BY SOME THAI TRADITIONAL PLANTS

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ABSTRACT

The aim of this study was to evaluate the efficiency of three types of Thai traditional plants on retardation the lipid oxidation reaction in pork. The extracted solution from traditional plants such as Garcinia cowa Roxb Phyllanthus emblica and Acacia Concinna DC were prepared by 7 methods and analysed total phenolic compound content and IC_{50}. An extracted solution were treated in fresh pork samples such as pork loin and pork blade shoulder and those samples were stored in freezer cabin of refrigerator. The pork samples were evaluated the lipid oxidation reaction by analysis of malondialdehyde content in the 7 , 14, 21 and 30 days of storage time. The results presented the leaves of plant that boiled in water for 30 mins gave the extracted solution with total phenolic compound content in the range of 946.2 ± 58.37 - 7342.4 ± 122.89 mg/100g. Phyllanthus emblica showed the maximum content of total phenolic compound, with IC_{50} as 4.41 mg/ml. All extracted solution of plants reduced the lipid oxidation reaction within 30 days of storage. Phyllanthus emblica extracts showed the best efficiency on retardation lipid oxidation in pork samples.

Keywords: Lipid Oxidation, Pork, Retardation, Traditional plants

INTRODUCTION

Lipid is the component in food that effects on flavor and texture of foods. On the otherhand, lipid also be oxidized and cause deterioration of food such as off flavor , texture change, color modifications and shorten shelf life[1]. Lipids can be oxidized by both enzymatic and nonenzymatic mechanisms[2]. Auto- oxidation reaction generally involving three main steps: as initiation step is the abstraction of hydrogen atom adjacent to a double bond in an unsaturated molecule of fatty acid to give an alkyl free radical. The propagation reaction which the reactive radical react with di-oxygen to form an unstable peroxyl free radical is the second one. The last step is a termination process, which corresponds to the combination of two radical species leads to many products as ketones, ethers, alkanes and aldehydes[3]. The protection method for lipid oxidation reactions in foods were studied especially in the meat industry. In general, food additives, food ingredients with antioxidant potential were used ,however the safety of additive substance must awareness for consumer. Now the natural antioxidants were interested. The natural sources such as fruits, vegetables, seeds and spices were studied and guide to preserve lipid oxidation[4-8]. For example as Rosemary compose of essential oils as antioxidants has been used in a meat products including refrigerated beef[9-10], frozen pork patties [11-12]or frankfurters[13]. The spices as cinnamon or clove has been prove to decrease lipid oxidation as effectively as certain synthetic antioxidants in cooked meat products[14-17]. However, the using plant leaf especially about traditional plant has no details to use for protect lipid oxidation, so the aim of this work was to evaluate an extracted substance from Thai traditional plants and applied to pork for reduce lipid oxidation reaction. From above details about the efficiency of some plant that contains many important antioxidant. There are many plants such as *Garcinia cowa* Roxb., *Phyllanthus emblica* and *Acacia*

Concinna DC which were interested in this work. The report from many papers refered that the *Garcinia cowa* Roxb which its leaf used as food especially Thai food as Kang Moo Chamuang. The *Garcinia cowa* Roxb leaf has sour taste since its contain many organic substance such as vitexin, orientin[18],beta-sitosterol, xanthone[19], cowanin ,cowanol , norcowanin, cowagarcinone A-E[20] and mangostinone[21]. Many parts of *Garcinia*. cowa was also used in traditional folk medicine. The barks, latex and roots have been used as an antifever agent while the fruits and leaves have been used for indigestion, improvement[22]. *Phyllanthus emblica* L.is a plant that its fruit has sour taste and contains many substance such as astragalin, glucogallin, gallic acid, digallic acid, ellagic acid, chebulagic acid, chebulinic acid, kaempferol, quercetin and ascorbic acid[23-24]. However, there was no report to use its leaf for food. The fruit of *Phyllanthus emblica* L is used as a traditional medicine in South Asian countries, such as it has been used as a treatment for glucose intolerance, cerebral insufficiency, hyperthyroidism, and mental disorders, and for the prevention of atherosclerosis[25-26], hyperlipidemia[27],diabetic cataracts[28]. Finally, *Acacia Concinna DC.* also used as folk medicine for cure skin , tendon and eating behavior[29]. An important compounds found in plant such as saponin[30-31], methylsalicylate, palmitic acid and linoleic acid[32]. From above details ,their leaves could be used as natural antioxidant in reduce the lipid oxidation in pork.

MATERIALS AND METHOD

Materials

Plant samples: *Garcinia cowa* Roxb., was purchased from fresh market in Rayong province, Thailand. *Phyllanthus emblica* and *Acacia Concinna DC* were brought from fresh market in Nakhornraschasrima province, Thailand.

Fresh pork samples: Pork loin and pork blade shoulder were purchased from Foodland supermarket, Bangkok ,Thailand.

Chemicals and Instrument

Sodium hydroxide (AR grade) was purchased from BDH. Ethanol (AR grade) , Methanol(AR grade) , were purchased from Acros. Folin chio caltuae reagent (AR grade), standard gallic acid (AR grade) were purchased from Fluka.White spiritual liquor (Liquor Distillery Organisation Excise Department) was purchased from Lotus super market in Bangkok. Folin-Ciocalteu reagent, sodium carbonate(AR grade) , Gallic acid(AR grade), 2, 4-dinitrophenylhydrazine(ARgrade) were purchased from Sigma Chemical Co.,Ltd(St.Louis, Mo,USA). DPPH (AR grade from Fluka) ,BHT(AR grade) ,1,3,3-tetramethoxypropane(AR grade), Ascorbic acid(AR grade)were purchased from Fluka. 2-thiobarbituric acid(AR grade) ,Trichloroacetic acid(AR grade),Thiourea(AR grade) were purchased from Carlo Ebra.

General Procedure

PART 1. Preparation of extracted solution from plants samples

All plants leaves were separated from stem and washed with tap water, dried at room temperature. Dried leaves were treated by 7 methods as the following :

Method 1. (modified from Sun-Ja Kima.et al.2007)

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 200 ml water. Then samples were heated 60^{o}C for 2 hrs. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to dry by rotary evaporator (Buchii rotavapor).The pure water was added into the residue and collected for future use.

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Method 2. (modified from Sun-Ja Kima.et al.2007)

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 200 ml of 0.1 N. sodium hydroxide solution. Then samples were heated 60^{o}C for 2 hrs. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to left approximately as 5.00 ml.Then 15 ml. of 95% ethanol was added to the residual liquid and the mixed solutions were left overnight for 1 night. The ethanol was evaporated out by rotary evaporator.

Method 3. (modified from Yuan, Y.V. &Walsh, N.A. 2006)

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 100 ml of pure ethanol and left for 48 hrs. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to dry by rotary evaporator.

Method 4. (modified from Bonilla, E.P.,et al.2003)

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 100 ml of 2% hydrochloric acid solution and left for 24 hrs.. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to dry by rotary evaporator.

Method 5 (modified from Wang.L,& Weller.C.L.2006)

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 200 ml water.Then samples were boiled for 30 mins. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to dry by rotary evaporator. The pure water was added into the residue and collected for future use.

Method 6 (modified from Wang.L,& Weller.C.L.2006)

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 200 ml water.Then leaves samples were crushed and pressed to get a viscous solution. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to dry by rotary evaporator. The pure water was added into the residue and collected for future use.

Method 7. (modified from Hayek, S. A., et al.2013).

Plant leaves were chopped into small pieces , weighed 5.xxxx g and mixed with 200 ml of white spiritual liquor ,covered with lid and left for 7 days.. The residual plants were filtered out an collected the filtrate.The filtrates were evaporated to dry by rotary evaporator.

PART 2. Evaluation on the quality of extracted solution

2.1 Analysis of Total phenolic compound content

The analysis method modified from Pavel Stratil, et al.,(2006),the clear filtrate from part 1 were pipetted 0.4 ml and mixed with 2 ml of 10 % Folin Ciocalteau reagent and 1.6 ml of 7.5 % Na_2CO_3 and kept at room temperature for 30 mins. The mixing solution was measured an absorbance at 765 nm by Ultraviolet Visible Spectrophotometer(UV -VIS Shimadzu Model UV100) and calculated the Total phenolic compound content as gallic acid equivalent.

2.2 Analysis of IC_{50}

The analysis method modified from Ganhão, R., et al.(2013), the clear filtrate from part 1 were pipetted 600 µL mixed with 600 µL of 0.1 mM DPPH then measured an absorbance of the complex color from the reaction at 517 nm. The inhibition power was calculated by

comparing with standard BHT solution. Then those extracted solutions from part 1 were prepared as 5 concentration, then 0.1 ml of each solution was pipette and put into test tube.The 0.1 mM of DPPH 3.9 ml was added to the tube and left at room temperature in the dark cabin for 30 mins and measured an absorbance of the complex color from the reaction at 517 nm.

PART 3. Study on retardation of lipid oxidation in pork sample

3.1 Pretreatment on fresh pork

Fresh porks were cut into 10 small pieces(1.0x 1.0x1.0 cm , each piece approximately 5.xxxx g),and placed into the extracted solution from part 1 for 10 mins. All samples were stored in the freezer cabin of refrigerature for 1 month. The pork samples were analysed malondialdehyde as an index of lipid oxidation.

3.2 Analysis of Malondialdehyde content

The frozen pork sample was placed at room temperature before the analysis. Then pork sample was chopped into small pieces , and weighed 1.xxxx g. The 10 ml of 5% TCA was added to pork sample and ultrasonic for 10 mins. The filtrate of each sample was collected for analysis malondialdehyde content[39].Then 0.5 ml of filtrate was pipetted and mixed with 1.0 ml of 0.5% w/v 2-thiobarbituric acid in 20% TCA. The mixture was heated at 95 ^{o}C in constant temperature water bath for 30 min. and immediately cooled in ice bath to room temperature. The mixing solution was centrifuged at10,000 g x for 5 min, and the absorbance of supernatant was recorded by Ultraviolet Visible Spectrophotometer at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances (TBARS) and 1,3,3-tetramethoxypropane was used as standard. The content of TBARS was calculated by comparison with the standard curve, and the level of lipid peroxides was expressed as malondialdehyde content in unit of mg per 100 gram of wet vegetables.

Statistical Analyses

All determinations were carried out at least in five replicates and values were averaged. For all statistics, ANOVA and Microsoft Excel were used for calculate and graph presentation in this work.

RESULTS AND DISCUSSION

After study the extracted method from leaves plant of all rural plants by 7 methods; method 1-4 were the chemical method and the last 3 method were the physical method. The results presented in figure 1. It showed that an important substance as total phenolic compound content in each leaf plant depend on the method of extraction. The quantity of total phenolic compound from each plant had the same trend by the method of extraction by method7 or m7 could extract the highest content of important substance in the range of 1230- 9846 mg/100g. The method 7 used white spiritual liquor in extraction and using a long time for 7 days in fermentation, this solvent could dissolve an organic compound in plant leaf better than common solvent as water, base or acid. Thus, using the m 7 was the best method to extract an important substance out off plant fiber. However, the method 5 which was simple treatment on plant leaf by boiling plant in water for 30 mins. also showed the high total phenolic compound content in the range of 946.2 ± 58.37 - 7342.4 ± 74.99 mg/100g of fresh plant leaf. This method was simple and easy with practical in the true life and also gave a high important substance ,thus the method 5 was very interesting to apply in next part.

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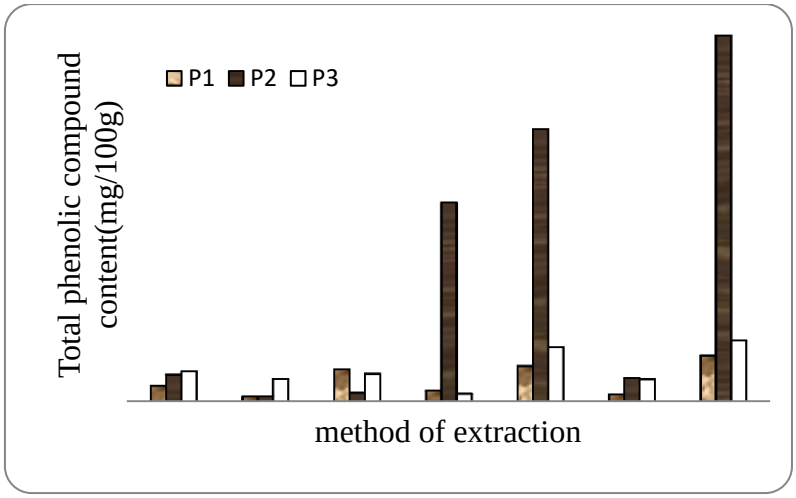

Figure. 1 Total phenolic compound content in leaves plant samples

Note: P1 = *Garcinia cowa* Roxb
P2 = *Phyllanthus emblica*
P3 = *Acacia Concinna DC.*
m1 = method 1 m2 = method 2 m3 = method 3 m4 = method4
m5 = method 5 m6 = method 6 m7 = method 7

From this work revealed that P2 or *Phyllanthus emblica* leaf contained maximum total phenolic compound approximately as 9846.8± 45.87 mg/100g in extracted solution from leaf by m7 and 7324.45 ± 74.99 mg/100g in extracted solution from leaf by m5. The method of extraction effective compound from plant also support the details about plant species which contain various water-soluble active compounds that could be extracted by using water as a solvent or directly used as a pure extract[40].

The results in the analysis of antioxidant inhibition that showed in term of IC50 as in table1.

Table 1. IC_{50} of traditional plants

Plant samples	IC_{50}(mg/ml)
Garcinia cowa Roxb	7.71
Phyllanthus emblica	4.42
Acacia ConcinnaDC	6.21

From IC_{50} value also showed that *Phyllanthus emblica* leaf has the highest efficiency on antioxidation, which related to the highest value of total phenolic compound content of this plant. This result also supported the report from Patel & Goyal[26],that referred the important substance in *Phyllanthus emblica* fruit. However, others traditional plant as *Acacia Concinna DC.* was also interesting too, but it is a rare traditional plant.

The above results gave the good sign to take the extracted solution from each plant on study the effect on retardation lipid oxidation with pork.The fresh porks were treated with extracted solution from the leaves of all three plants by method 5 as the reason for that it is safe to use in cruisine Thai cooking. The malondialdehyde was analysed in one month by measurement in a period of 7 , 14, 21 and 30 days.The results showed in figure 2 and 3.

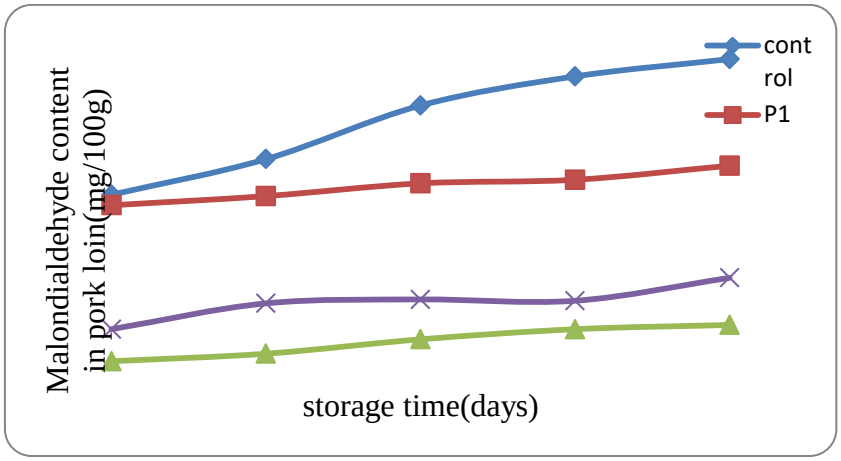

Figure. 2 Malondialdehyde content in storage pork loin

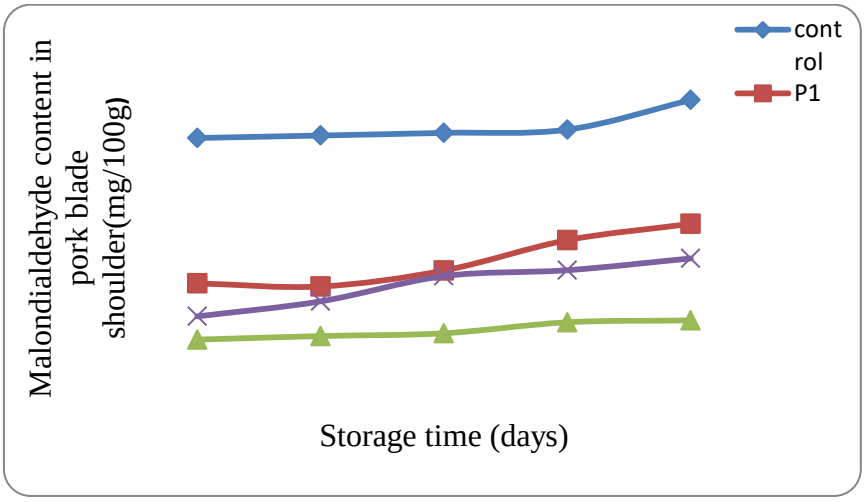

Figure 3. Malondialdehyde content in storage pork blade shoulder

Note: P1 = *Garcinia cowa* Roxb
P2 = *Phyllanthus emblica*
P3 = *Acacia Concinna DC.*

The above figure 2 and 3 showed the same trend that each extracted from leaves of plant could reduce an occurrence of lipid oxidation reaction in pork, because malondialdehyde content in pork after treatment with extracted solution lower than control pork. It was noticed that the malondialdehyde content depend on the part of pork, from this experiment the value of malondialdehyde in pork blade shoulder higher than in pork loin. This concern that in pork blade shoulder is full of higher lipid content than pork loin, so this also the higher possibility to oxidation as referred by McCarthy, T. L et al. 2001. However, this work revealed that P2 which was the extracted solution from *Phyllanthus emblica* had the highest effective to reduce the auto lipid oxidation in both part of pork. This support the experiment from part 2, the extracted solution from *Phyllanthus emblica* contained the highest value of total phenolic compound content and lowest IC_{50}, so this plant showed the best action on reduce the lipid oxidation. As above data, the effectiveness on protection lipid oxidation also related with an important substance found in plant. The new discovery knowledge from this work also proved that an extracted solution from each plant could retarded or reduced the lipid oxidation reaction within 30 days after storage in the freezer cabin of refrigerator. This

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knowledge can apply to protect the freezer food from lipid oxidation before storage , and still good quality and good for food export industry.

The using of leaf from traditional plant for treatment freezing food especially meat product may be the other choice to protect lipid oxidation of food. Finally, this work showed the utilities of rural plant leave to gain value added of plant. As describe above that the extracted solution from plant by boiling with water may gave the solution that contain the important active component from plant which can resists to many microbial and also shown inhibitory effects against pathogenic microorganisms. Antimicrobial agents from plants have been mostly used in food systems[41]. Then, the extracted solution from rural plant leaves showed the good activity to resist the lipid oxidation in meat product.

CONCLUSIONS

The leaf of rural plants which were treated as 7 methods gave the extracted substance that contained an important substance such as total phenolic compound in the range of 123.20 ± 12.33 – 9846.82 ±45.87 mg/100g , and IC_{50} in the range of 4.42-7.21 mg/ml.The total phenolic compound content depend on the method of treatment plant, and the method method 7 that used white spiritual liquor with fermentation for 7 days in extraction process gave the highest efficiency in extraction. However, the simple method as method 5 which boiling leaves in water for 30 mins also showed the high total phenolic compound content in the level of 946.2 ± 58.37 - 7342.4 ± 122.89 mg/100g. Since ,the simple treatment with hot water easy to practice for all people who interesting to treatment with meat products, thus the method 5 was chosen to treat with the rural plant in this experiment. The *Phyllanthus emblica* showed the highest total phenolic compound with lowest IC_{50}. The pork samples (pork loin and pork blade shoulder) were placed into an extracted solution of each plant and kept in freezer of refrigerator for 30 days and analysed malondialdehyde content as an lipid oxidation index for 7 days period.The results revealed that all extracted solution from those rural plant could protect lipid oxidation in pork for almost 30 days.

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