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Effect of wet heating and pH on fatty acids of fresh coconut milk

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Abstract The types and amounts of fatty acid in coconut milk did not change on the addition of 10% citric acid or 10% sodium carbonate or on steaming at 100℃ for 20, 40 or 60 min, though the physical appearance altered. The types and amounts of fatty acid in fresh coconut milk was not changed following autoclaving at 116 and 121℃ for 15, 30 or 45 min, though the physical appearance changed. These results showed that coconut milk had desirable properties as its composition of fatty acids that was stable under steaming or the addition of an acid or a base.

Keywords: Fatty acids, Coconut milk, Wet heating

Introduction

Coconut milk is extracted from the kernel of mature coconut fruit (Cocos nucifera L.) and it is used in many kinds of foods in the Asian and Pacific regions (Chiewchan *et al.*, 2006). Coconut milk contains 230 kilocalories and is 67.5% water, 23.8% total fat, 5.54% carbohydrates, 2.29% protein and 0.70% ash. The main of fat is saturated fat (88.64%) but it contains a lower amount of unsaturated fat as 4.20% monounsaturated fat and 1.05% polyunsaturated fat (Conde Nast, 2018).

Tinchan *et al.* (2015) reported that the volatiles compounds of canned coconut milk changed after processing at 121℃ for 5 min followed by storage at ambient temperature for 6 months. After 2 months, high levels of alcohols, acids and lactones were identified and after 5 months, the levels of lactones, short chain free fatty acids, 3-methyl-2(5H)-furanone, and phenol had greatly increased. Increases in acetic and butyric acids contributed to an off odor. Pai *et al.* (1979) reported on heating coconut oil, 180℃, 48 hr, with three different heat treatments (heating under vacuum, heating in air and heating with water

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added at 1% by weight). The unheated control had small amounts of free fatty acids (C6:0 - C14:0) while heating in a vacuum increased the amounts of all free fatty acids but did not change the qualitative pattern. Heating in air increased the amounts of all free fatty acids and produced five fatty acids not detected in the vacuum sample. There was a 28-fold increase in decanoic acid (C10:0) from the decomposition of the 8-hydroperoxide of monounsaturated oleic acid; octanoic acid (C8:0) was expected to occur die to the decomposition of the 9-hydroperoxide of oleic acid. The sample with water increased amounts of all of C6:0 – C14:0, especially, octanoic acid that increased 70-fold, while the others increased 30–40 fold compared to heating in air. Saittagaroon *et al.* (1984) identified nine new aroma constituents of roasted coconut meat. Saturated delta-C8:0, C10:0 and C12:0 lactones were the main components producing a strong characteristic sweet and nutty aroma. Six pyrazines, two furans and two pyrroles were identified as the major Maillard reaction products.

Many kinds of food contains coconut milk, such as galangal root soup and also mussaman curry (having tamarind to give it a sour taste because it is high in tartaric acid according to El-Siddig *et al.* (1999), so the fatty acids of coconut milk may change while cooking or with the addition of other ingredients, though information is still lacking in this field. The aim of the current research was to study the effect of a wet heating process to determine whether an acid or a base that added to the fatty acid in fresh coconut milk.

Materials and methods

Fresh and mature coconuts (age 10 – 12 months) were obtained from a local market in Thailand. The fruits were subjected to deshelling, paring and removal of water. The coconut kernel was disintegrated using an electric coconut grater. The grated coconut kernel was subjected to expelling in a screw press to extract the coconut milk.

PH and time treatments at 100 ℃

The experiment was conducted using a 3×3 factorial in randomized complete block design (RCBD) with two replications. The first factor was pH containing three levels: 1) 10% citric acid added 2) 10% sodium carbonate added and 3) no acid or base added. The second factor was time, consisting of three levels of 20, 40 or 60 min. The coconut milk was separated into three parts— for the addition of either citric acid, sodium carbonate or a sample without adding either the acids or bases according to the experimental plan. The separate mixtures were poured into glass bottles and sealed with tight lids. The

samples were placed in a steamer at 100℃ according to the time of each treatment and then were removed and allowed to cool at room temperature. All samples and untreated fresh coconut milk were kept at -20℃ until they were analyzed for fatty acids using gas liquid chromatography with the TMC-05 in-house method of the Compendium Methods for Food Analysis Thailand (2003), for pH using a pH meter (Brand: Sartorius, Model: Docu) and for color using a spectrophotometer (Spectraflash 600 plus, Data-color International, USA) which was recorded as: L* = lightness (0 = black, 100 = white); a*(-a* = greenness, +a* = redness); and b*(-b* = blueness, +b* = yellowness). Data were subjected to analysis of variance of the factorial in RBCD and Duncan's new multiple range test for inspection of mean differences at a significance level of 0.05, using the SPSS version 12 statistical software (now a part of IBM Corp.; White Plains, NY, USA) and to a t-test: for paired samples for a means comparison of untreated fresh coconut milk and treated coconut milk at a significance level of 0.05.

Temperatures and time treatments over 100 ℃

The experiment was conducted using a 2×3 factorial in (RCBD) with two replications. The first factor was two levels of temperature (116 and 121℃). The second factor was three levels of time (15, 30 and 45 min). The fresh coconut milk was poured into glass bottles, and the lids were tightly closed. The samples were placed in an autoclave and steamed for the relevant time after which they were removed and allowed to cool at room temperature. All samples and the untreated fresh coconut milk were kept at -20 ° C until they were analyzed for fatty acid quantification. Data were subjected to analysis of variance of the factorial in RCBD and Duncan's new multiple range test at a significance level of 0.05 and to a t-test of paired samples for means comparison of untreated fresh coconut milk and treated coconut milk at a significance level of 0.05.

Determination of the fatty acids composition

Fat extraction and fatty acid methyl esters preparation

The fat of coconut milk was extracted using 10 g of sample added with 30 ml of 4:1 hexane (98.67% purity, Fisher,USA): acetone (99.0% purity, J.T. Baker,USA) and placed in a shaker (GFL 3016, Germany) for 30 min. Then, the supernatant was passed through filter paper (Whatman No.1, Merck, Germany) and sodium sulphate anhydrous (99.5% purity, BDH, England) for water absorption. The residue of coconut milk was added with the same solvent

and the process was repeated. The solvent was evaporated in a rotary evaporator (Rota vapor R-200, Buchi Syncore Analyst, Switzerland) at 400ºC and 400 millibar. The fat of the coconut milk was added with 5 ml of 0.5 M potassium hydroxide (85 % purity, BDH, England) in methanol (Merck, Germany), then placed in a water bath (Heto SBD 50, Denmark) at 100ºC for 5 min and then left to cool. Immediately after, it was added with 2 ml of boron trifluoride methanol complex 20% solution (BDH, England), then placed in the water bath at 100ºC for 15 min and again left to cool. It was immediately, added with 10 ml of saturated sodium chloride (99.5% purity, Merck, Germany) and the supernatant was extracted with petroleum ether (35– 600ºC boiling point, J.T. Baker, USA) and this solvent was evaporated in the rotary evaporator at 400ºC and 400 millibar. Next, the residue was added with 1.0–3.0 ml chloroform (99.0-99.4% purity, BDH, England) and passed through filtered through a 0.45 um, 13 mm nylon disposable syringe filter.

Gas chromatography

The fatty acid methyl esters were separated in a gas chromatograph (Varian CP-3800, USA) equipped with a split injector (100:1), column (Agilent J&W HP-88 capillary column, 100 m × 0.25 mm i.d., 0.20 µm film thickness) and an FID detector. The initial temperature of the column was 140 ℃ for 5 min, programmed to rise to 240 ℃ at 4 ℃/min and held for 20 min with an injector volume of 1 µl and a detector temperature of 260 ℃. The carrier -gas was helium at a flow rate of 1.7 ml/min. The fatty acids were identified by comparison of the retention times of the sample with those of the standards and by spiking. In total, 37 fatty acid standards (SulpecoTM 37, USA) were used to verify the identity and the accuracy of the method. Quantification was carried out based on area percentages.

1. Percentage of fat = (M2 – M1)×100/W where M2 is the weight (grams) of round bottle flask and fat, M1 is the weight (grams) of round bottle flask and W is the weight (grams) of sample.
2. Area percentage of fatty acid = 100 × Ax /At - Ats where Ax is the area counts of fatty acid X, At is the total area counts for the chromatogram and Ats is the area counts of the internal standard (tricosanoic acid methyl ester (99.8% purity, Fluka, USA),.
3. Fatty acid (grams) / 100 grams sample = Ft × Fc × Fa /100 where Ft is the total fat (grams) in 100 grams sample, Fc is a constant (0.942) and Fa is the fatty acid area percentage.

Results

Effect of pH and time on steaming at 100 ℃

The fresh coconut milk was an opaque, white color, mildly acidic and mildly sweet with 5-7 ºBrix total soluble solids, containing approximately 19 – 21% fat based on acetone and hexane extraction. The colors of the treated and unsteamed samples are shown in Figures 1-3. The three samples with citric acid added followed by 20, 40 or 60 min of steaming time had pH levels in the range 2.3 – 2.45 and were very sour. The texture of the acid samples was liquid. The color was opaque white but only at the bottom of the bottles was there an appeared the change of brown to red which decreased in L* and b* but increased in a* which compared to unsteamed coconut milk. The samples which no acid or base were added, the pH level was ranged between 5.6–5.94 after 20, 40 or 60 min of steaming time and the samples showed a mild sweet taste. The color was more opaque white as there were decreased in the values of L*, a* and b* which compared to the unsteamed coconut milk. The three samples with sodium carbonate added and then steamed for 20, 40 or 60 min of steaming time had high pH values in the range of 10.18–10.20 and very bitter taste. The color was opaque brown due to the decreasing values of L*, a* and especially of b* compared to the unsteamed coconut milk. These added base had a mild curded texture and turned to mild brown color immediately after the base was added and became a dark brown color after steaming.

The results of the fatty acid composition analysis for pH and time are shown in Figure 4 and 5. There was no interaction between the two factors. The main effect of the three levels of pH and steaming time did not change the eight types of fatty acids ($p > 0.05$) of the treated steamed samples. The comparison of mean values of the fatty acids in the fresh untreated samples and the treated samples is shown in Table 1. There were no significant differences between the paired comparisons of the eight types of fatty acids.

Effect of high pressure and autoclave time

The results of the fatty acid compositions showed the main temperature and time effect (Figure 6 and 7.) with non-interaction between the two factors. The main effect of the three levels of temperature and steaming times did not change the eight types of fatty acids ($p > 0.05$) of treated samples. The comparisons of the mean values of fatty acids in the fresh, untreated samples and treated samples are shown in Table 2. There were no significant differences in the paired comparisons of the eight types of fatty acids.

Discussion

The oil content in fresh coconut milk was approximately 19 – 21% fat that was low compared to the 30.34% fat reported by Jeganathan, 1970, probably because of different species of coconuts. As some foods have a sour taste from the addition of lime or tamarind, citric acid was used in this research. Citric acid is a weak organic acid with the formula $C_6H_8O_7$. It has an E number of E330 within the European Union and as a food additive by the Food Chemicals Codex. It occurs naturally in citrus fruit but may also be prepared from the fermentation of molasses. It is used as an antioxidant, preservative, acid regulator and flour improver (Rademaker, 2003). However, some foods contain coconut milk and have a higher pH, so sodium carbonate is used. Sodium carbonate, Na_2CO_3, is the sodium salt of carbonic acid, is soluble in water and forms a strongly alkaline aqueous solution. It too is a food additive (E500) and is used as an acidity regulator. The red color of the acid samples may have been due to the Maillard reaction of the reducing sugar and amino acids or of other nitrogenous compounds with heat as a catalyst because coconut milk has 3.0% protein such as globulins and albumins (Jeganathan, 1970; Tangsuphoom and Coupland, 2008). Jayalekshmy and Mathew (1990) reported that sucrose was the most common carbohydrate in coconut milk approximately 7.50 g/100 g dry weight. The quantity of sucrose in 100℃ steamed coconut milk after 1 hr was 2.28, 0.00, 2.94 and 2.66 g /100 g of milk, respectively, in untreated fresh coconut milk, acid-added, steamed coconut milk, nothing-added, steamed coconut milk and sodium carbonate-added, steamed coconut milk. The results showed that the addition of acid with heating the coconut milk may have resulted in hydrolysis reactions to reduce all the sucrose molecules to glucose and fructose (Siddiqui, 2010). With the samples where no acid or base was added, the pH level was in the range 5.6 – 5.94 after 20, 40 and 60 min of steaming time and the samples had a mild sweet taste, with a more opaque, white color as there was a decrease in the values of L*, a* and b* compared to the unsteamed coconut milk. The texture of these samples was curd-like because some heat-labile proteins had been denatured and had coagulated rapidly upon heating to 80℃ (Steinkraus *et al*., 1968). The brown color of base-added samples may have been due to a caramelizing reaction. This was proven by preparing two samples of: 1) 2% glucose mixed with 10% sodium carbonate and 2) 2% sucrose mixed with 10% sodium carbonate and heating them on a gas stove. The color of the glucose sample changed to yellow and brown while the color of the sucrose sample was not changed as the glucose was destroyed by the sodium carbonate, especially with the higher pH of 10 and catalysis with heat (Villamiel *et al*., 2006).

This type of coconut milk contained eight types of fatty acid according to Metcalfe *et al*. (1966), who reported that coconut oil contained: C8:0, 4.6%; C10:0, 5.3%; C12:0, 47%; C14:0, 20%; C16:0, 9.8%; C18:0, 1%; C18:1n9c, 11%; and C18:2n6c, 1.1%. Caprylic acid ($C_8H_{16}0_2$, C8:0) or systematically, octanoic acid, is a saturated, medium fatty acid, found as the first fatty acid and making up the sixth quantity of approximately 0.5 – 1.46% in coconut milk. Octanoic acid is found in the milk of mammals and is a minor component of coconut oil and palm kernel oil. It is an antibacterial agent, a human metabolite and an *Escherichia coli* metabolite (National Center for Biotechnology Information, 2021). Capric acid ($C_{10}H_{20}0_2$, C10:0) or systematically, decanoic acid, is a saturated, medium fatty acid and the fifth quantity found constituting approximately 0.94 – 1.22% in coconut milk. It is an antibacterial agent, an anti-inflammatory agent, a human metabolite, a plant metabolite and an algal metabolite (National Center for Biotechnology Information, 2021). Lauric acid ($C_{12}H_24O_2$, C12:0) or systematically, dodecanoic acid, is a saturated, medium fatty acid, with the highest quantity found being approximately 9.43–10.36% in coconut milk. Lauric acid is found in laurel oil, palm kernel oil and in human breast milk (6.2% of total fat), cow's milk (2.9%), and goat's milk (3.1%) (Beare-Rogers *et al.,* 2001). Lauric acid increases the level of high-density lipoprotein (HDL) (Mensink *et al.,* 2003). Myristic acid ($C_{14}H_{28}O_2$, C14:0) or systematically, tetradecanoic acid, is a saturated fatty acid and the second quantity found being approximately 3.77– 4.14 % in coconut milk. Myristic acid is found in nutmeg, palm kernel oil, butter fat and is a minor component of many other animal fats. (Beare- Rogers *et al.,* 2001). Palmitic acid ($C_{16}H_{32}0_2$, C16:0), or hexadecanoic acid in IUPAC nomenclature, is a saturated, fatty acid and the third quantity found being approximately 1.17–1.92% in coconut milk. Palmitic acid is found in palm oil and palm kernel oil, in butter, cheese, milk and meat. It is a plant metabolite, a Daphnia magna metabolite and an algal metabolite (National Center for Biotechnology Information, 2021). Stearic acid ($C_{18}H_{36}O_2$, C18:0), or octadecanoic acid in IUPAC nomenclature, is a saturated, fatty acid and the seventh quantity found being approximately 0.48–0.63% in coconut milk. It is found abundantly in animal fat (up to 30%) and vegetable fat (typically <5%) (Beare-Rogers *et al.,* 2001). Oleic acid ($C_{18}H_{34}O_2$, C18:1n9c) or cis-9-octadecenoic acid is a monounsaturated, omega-9 fatty acid. Oleic acid is essential to the human body. It lowers the risk of heart disease and helps in cancer prevention (Lopez *et al*., 2010). It was the fourth quantity found being approximately 0.95 – 1.31% in coconut milk. Oleic acid is the most abundant fatty acid in human adipose tissue (Kokatnur *et al.,* 1979) and is found abundantly in olive oil (55–-83%) (Beltrán *et al.,* 2004). Linoleic acid (C18H3202, C18:2n6c), or (9Z, 2Z)-9,12-octadecadienoic acid in IUPAC

nomenclature, is an essential polyunsaturated fatty acid. It is a plant metabolite, a Daphnia galeata metabolite and an algal metabolite (National Center for Biotechnology Information, 2021). It was the lowest quantity found being approximately 0.19 – 0.29% in coconut milk. No evidence was found of caproic acid (C6H12O2, C6:0), or hexanoic acid in IUPAC nomenclature, that was reported by Banzon and Resurreccion (1979) with the quantity of C6 being the lowest value of approximately 0.57 – 0.63% in coconut oil in the Philippines.

In the currentis study, the fatty acid compositions in the coconut milk were not changed on the addition of citric acid or sodium carbonate or with steaming time which agreed with the research of Banzon and Resurreccion (1979) who observed no change in the fatty acid composition of coconut oil from four methods: 1) solvent extraction of dried, grated coconut kernel with ethyl ether in a Goldfisch Extractor;, 2) fermentation of diluted milk with tap water 1:4 (v/v) and allowed to stand at 30 – 34 ℃; 3) freeze-thawing of the washed floating cream; and 4) heat rendering of cream using the kitchen method as the cream was heated resulting in free coconut oil. Nevin and Rajamohan (2006) also found that the fatty acid composition of copra oil from dried coconut kernel was not different to virgin coconut oil obtained using the wet process and temperature control. The fatty acids of coconut oil are influenced by the growing conditions (Balleza and Sierra, 1972); season (Naresh Kumar and Balakrishna, 2009) and genotypic variations (Naresh Kumar *et al*., 2000; Naresh Kumar *et al*., 2004; Laureles *et al*., 2002).

There was not much fat in the coconut milk and approximately 70% of triglycerides in coconut oil are lower chain saturated fatty acids known as medium- chain fatty acids (MCFAs). The MCFAs of coconut oil are different from vegetable oils with lauric acid at 49.10–52.10% (Banzon and Resurreccion, 1979) and more importantly, coconut oil contains only 11% monounsaturated fatty acids and only 1.1% polyunsaturated fatty acid (Metcalfe *et al*., 1966), making it stable because oxidation occurs in the unsaturated fatty acids, especially, as highly unsaturated fatty acids are more easily oxidized (Matsushita, 1990). Benedict *et al*. (1975) found that the high polyunsaturated levels in meat increased its deterioration. However, keeping the product at room temperature may cause changes in the fatty acid or volatile compounds or both, which should be further studied because Tinchan *et al*. (2015) found that the volatiles compounds of canned coconut milk changed after storage for 6 months due to lipid oxidation and lipolysis reactions as short- and medium-chain fatty acids were observed in high amounts. Monounsaturated acid as oleic acid is oxidized to form octanal, and oxidation of polyunsaturated linoleic acid was identified.

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