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Production of Pickled Chinese Olive (*Canarium Album*) Fruit

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Abstract.
Chinese olive (*Canarium album* L.), a plant in the Burseraceae family, is widely cultivated in Vietnam. The fresh fruit has the organoleptic characteristics of strong bitter and astringent tastes, and then tastes fragrant, sour and sweet after being chewed for a longer time. An attempt explored a lactic fermentation from *Canarium album* by focusing on the effect of different parameters such as blanching time and temperature as pre-treatment, salt concentration, fermentation time to total phenolic compounds, total flavonoids, total triterpenoids, trolox equivalent antioxidant capacity of pickled *Canarium album***.** Experimental results revealed that blanching raw *Canarium album* in water heated at 95^{o}C in 10 seconds, 6% salt in 12 days of fermentation was appropriated to get a pleasant pickled *Canarium album* quality. *Canarium album* pickles are considered as one of the health supplements.

Keywords: *Canarium album, lactic fermentation, salt, blanching, total phenolic compounds, total flavonoids, total triterpenoids, trolox equivalent antioxidant capacity*

1. INTRODUCTION

Canarium L. belongs to the family of Burseraceae Kunth. The members of the genus Canarium L. consist of medium to large buttressed trees up to 40-50m tall, or rarely a shrub. The barks are greenish grey, fawn or light yellow brown that are usually smooth, scaly or dippled with many small lenticels. Outer bark are thin while the inner barks are pinkish brown or reddish brown, laminated, soft and aromatic with a clear sticky or rarely oily exudate. The stems are usually terete (R. Mogana and C. Wiart, 2011). The dried fruit of chinese olive or Canarium album is used to treat bacterial and viral infections, inflammation, poisoning and for detoxification (Zhiyong H et al., 2008). Mature Chinese olive fruit is a fusiform drupe and yellowish green. The fresh fruit has the organoleptic characteristics of strong bitter and astringent tastes, and then tastes fragrant, sour and sweet after being chewed for a longer time. Some fresh fruits of Chinese olive are edible; however, they are normally pickled before eating, and most of them are generally processed in the food industry to beverages, candy and conserves (Chiung-Tsun Kuo et al., 2015). Some pharmacological functions such as hepatoprotective, antimicrobial and antivirus properties of Chinese olive fruits have been demonstrated (Tamai M et al., 1989; Fu BH, Zhou XH, 1997; Kong GX et al., 1998). Chinese olive fruit may ameliorate metabolic dysfunction in diabetic (Yu-Te Yeh et al., 2017). Zhang and Lin indicated that tannins extracted from the leaves, twigs and stem bark of Chinese olive exhibited very well radical scavenging activity and ferric reducing power (Zhang LL, Lin YM., 2008). Chinese olive fruit is rich in phenolic compounds and triterpenoids (He Z, Xia W., 2007, 2008, 2009). The major sugars and organic acids in chinse olive were glucose, fructose, and malic acid (Qiang Chang et al., 2017).

Lactic acid bacteria convert the carbohydrate contents of this fruit into lactic acid, which decreases the pH of the pickled products ensuring stability. Lower pH value restricts the growth of spoilage flora and pathogenic bacteria. These bacteria improve the human intestinal microbial balance and enhance health by inhibiting the growth of pathogens such as Escherichia coli, Salmonella and Staphylococcus (Ohmomo et al. 2000, Ross et al. 2002). They are often considered as probiotic, benefi cial for human health and active in lowering the serum cholesterol level (Kaur et al. 2002). They also stimulate immune responses and prevent tumour formation by inhibiting carcinogenic compounds in the gastro-intestinal tract through reducing fecal bacteria enzyme activity (Nakphaichit et al. 2011) or breaking down certain enterotoxins (Bernardeau et al. 2006). The fermented vegetables or pickle products made with characterized probiotic strain confirms the supplementation of probiotics to the consumers (Chaiyavat Chaiyasut, 2018).

Canarium album is an underutilized vegetable crop and still now there is very limited research available regarding to processing of this vegetable into value added product. The *Canarium album* vegetable, which typically has high fermentable sugar composition, could be exploited as a substrate for lactic fermentation. *Lb. fermentum* plays an important role in the *Canarium album* fermentation. Therefore, we utilized this vegetable as subtrate for lactic fermentation. We focused on the effect of different parameters such as blanching time and temperature as blanching temperature and time, salt concentration, fermentation time to total phenolic compounds, total flavonoids, total triterpenoids, trolox equivalent antioxidant capacity of pickled *Canarium album*.

2. MATERIAL & METHOD

2.1 Material

Chinese olive (*Canarium album)* fruits were cultivated and collected from Bac Lieu province, Vietnam. They must be cultivated following VietGAP without pesticide and fertilizer residue to ensure food safety. After harvesting, they must be conveyed to laboratory within 8 hours for experiments.

Figure 1. Chinese olive (*Canarium album)*

2.2 Research method

2.2.1 Effect of blanching temperature and time to total phenolic compounds, total flavonoids and total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive (Canarium album)

Canarium album fruits were pre-treated by blanching in water containing 0.5% $CaCl_2$ with different time and temperature (100^{o}C in 10 seconds, 95^{o}C in 15 seconds, 90^{o}C in 20 seconds and 85^{o}C in 25 seconds). Effectiveness of blanching time and temperature in *Canarium album* fermentation was evaluated on value of total phenolic compounds (mg/g), total flavonoids (mg/g) and total triterpenoids (mg/g), trolox equivalent antioxidant capacity (mmol/mg)

2.2.2 Effect of salt concentration in fermentation to total phenolic compounds, total flavonoids and total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive (Canarium album)

Canarium album fruits were fermented with different salt concentration (2%, 4%, 6%, 8%). Effectiveness of salt concentration in *Canarium album* fermentation was based on value of total phenolic compounds (mg/g), total flavonoids (mg/g) and total triterpenoids (mg/g), trolox equivalent antioxidant capacity (mmol/mg)

2.2.3 Effect of fermentation time to total phenolic compounds, total flavonoids and total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive (Canarium album)

Canarium album were fermented with different fermentation time (3, 6, 9, 12 days). Effectiveness of fermentation time in *Canarium album* fermentation was based on value of total phenolic compounds (mg/g), total flavonoids (mg/g) and total triterpenoids (mg/g), trolox equivalent antioxidant capacity (mmol/mg).

2.3 Physicochemical, microbial, sensory evaluation

Total phenolic compounds (mg/g) in the extracts were determined using Folin–Ciocalteu reagent. The content of total phenolics was expressed as gallic acid equivalents (GAE). The spectrophotometer assay for the quantitative determination of flavonoid content (mg/g) was carried out. Total flavonoids (mg/g) of fruits were expressed as catechin equivalents. The total content of triterpenoids (mg/g) was determined as described previously (Chen Y et al., 2007). The content of total triterpenoids was expressed as oleanolic acid equivalents. Trolox equivalent antioxidant capacity (mmol/mg) was measured as described previously (Erel O, 2004). Trolox and antioxidant ability was expressed as TEAC value

2.4 Statistical analysis

Data were statistically summarized by Statgraphics Centurion XVI.

3. RESULT & DISCUSSION

3.1 Effect of blanching temperature and time to total phenolic compounds, total flavonoids and total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive *(Canarium album)*

Blanching is a short time heat treatment widely applied before processing (freezing, frying, drying, and canning) to inactivate deleterious enzymes and to destroy various microorganism present in fresh green vegetables. Commonly hot water blanching technique is applied in the food industries and particularly in the processing of green leafy vegetables (Prakash Kumar Nayaka et al., 2018).

Canarium album were pre-treated by blanching in water containing 0.5% $CaCl_2$ with different time and temperature (100^{o}C in 10 seconds, 95^{o}C in 15 seconds, 90^{o}C in 20 seconds and 85^{o}C in 25 seconds). Effectiveness of blanching time and temperature in *Canarium album* fermentation was evaluated on value of total phenolic compounds (mg/g), total flavonoids (mg/g) and total triterpenoids (mg/g), trolox equivalent antioxidant capacity (mmol/mg). Results were depicted in table 1. It's clearly noticed that blanching at 95^{o}C in 15 seconds was optimal for *Canarium album* fermentation. So we selected this value for next experiments.

Liu et al. (2008) indicated that the contents of total phenolics and flavonoids in Chinese Olive fruit were 280.5 mg GAE/g and 130.29 mg rutin equivalents/g, respectively, expressed on a dry weight basis of fruit. The phenolic compounds such as quercetin, rutin, naringin, catechins, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents because of their antioxidant activity (Cai Y et al., 2004; Cai YZ et al., 2006). Blanching to remove the testa of canarium may deactivate or reduce enzymes (Saltveit, M.E., 2000).

Table 1. Blanching temperature and time to total phenolic compounds, total flavonoids, total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive *(Canarium album)*

Blanching temperature and time	Total phenolic compounds (mg/g)	Total flavonoids (mg/g)	Total triterpenoids (mg/g)	Trolox equivalent antioxidant capacity (mmol/mg)
100oC, 10 seconds	170.48±0.01b	15.94±0.01b	275.38±0.02b	1.76±0.01b
95oC, 15 seconds	**179.63±0.03a**	**16.73±0.02a**	**286.19±0.01a**	**1.93±0.02a**
90oC, 20 seconds	164.17±0.02bc	15.68±0.00bc	273.41±0.00bc	1.71±0.03bc
85oC, 25 seconds	157.22±0.03c	15.32±0.02c	272.04±0.03c	1.65±0.00c

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2. Salt concentration (%) to total phenolic compounds, total flavonoids, total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive *(Canarium album)*

Salt concentration (%)	Total phenolic compounds (mg/g)	Total flavonoids (mg/g)	Total triterpenoids (mg/g)	TEAC (mmol/mg)
2	179.63±0.03c	16.73±0.02b	286.19±0.01c	1.93±0.02c
4	181.22±0.01b	16.84±0.00ab	294.31±0.03b	1.97±0.00b
6	**185.74±0.02a**	**16.95±0.01a**	**302.08±0.03a**	**2.03±0.01a**
8	182.30±0.00ab	16.90±0.03ab	299.10±0.02ab	2.00±0.03ab

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3. Fermentation time (days) to total phenolic compounds, total flavonoids, total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive *(Canarium album)*

Fermentation time (days)	Total phenolic compounds (mg/g)	Total flavonoids (mg/g)	Total triterpenoids (mg/g)	TEAC (mmol/mg)
3	185.74±0.02b	16.95±0.01b	302.08±0.03b	2.03±0.01b
6	193.02±0.01ab	17.45±0.03ab	309.37±0.00ab	2.09±0.03ab
9	207.18±0.01a	17.64±0.02ab	316.35±0.02a	2.14±0.02a
12	207.22±0.03a	17.70±0.01a	316.41±0.01a	2.15±0.00a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

3.2 Effect of salt concentration in fermentation to total phenolic compounds, total flavonoids and total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive *(Canarium album)*

In the pickling industry, salt has historically been used for directing the fermentation of cucumbers, radishes, and carrots (Thompson RL et al., 1979; Hudson JM et al. Fleming HP et al., 1995; Mcfeeters RF et al., 1993). Sodium chloride is an essential in food as it improves the preservative, technological and sensory quality of food (Brady M., 2002). NaCl is one of the most commonly employed agents for food conservation, allowing considerable increase in storage time by reducing water activity. *Canarium album* were fermented with different salt concentration (2%, 4%, 6%, 8%). Effectiveness of salt concentration in *Canarium album* fermentation was based on value of total phenolic compounds (mg/g), total flavonoids (mg/g) and total triterpenoids (mg/g), trolox equivalent antioxidant capacity (mmol/mg). Results were depicted in table 2. It's clearly noticed that 6% salt was optimal for *Canarium album* fermentation. So we selected this value for next experiments.

Once the vegetables have been brined and the container sealed, a rapid development of microorganisms is observed in the brine. The natural parameters that affect the microbial populations of the fermenting vegetables include the concentration of salt and temperature of the brine, the availability of fermentable materials and the numbers and types of microorganisms present at the start of fermentation. The rapidity of the fermentation is correlated with the concentration of salt in the brine and its temperature (Ray and Panda, 2007). Most vegetables can be fermented at 12.5 to 20º Salometer. If so, the sequence of LAB generally follows the classical sauerkraut fermentation. At higher salt levels of about 40º Salometer, the sequence is skewed towards the development of a homo-fermentation, dominated by Lactobacillus plantarum. At highest salt concentrations as 60º Salometer, lactic fermentation stops and if any acid is detected during brine storage, it is acetic acid, presumably produced by acid-forming yeasts which are still active at this salt concentration (Montet et al., 2006). Salting is an important step in vegetable fermentation. Sodium chloride concentration can range from 20 to 80 g/l during fermentation. LAB can tolerate high salt concentrations. This salt tolerance gives them an advantage over less tolerant species and allows LA fermentation that inhibits growth of non-desirable organisms (Rao et al. 2004). Salt induces plasmolysis in plant cells which releases mineral salts and nutrients from the vacuole and creates anaerobic conditions for proper growth of LAB around the submerged product (Gardner et al. 2001, Rakin et al. 2004, Wouters et al. 2013).

3.3. Effect fermentation time to total phenolic compounds, total flavonoids and total triterpenoids, trolox equivalent antioxidant capacity of pickled Chinese olive *(Canarium album)*

Naturally occurred lactic acid bacteria from the raw ingredients play an important role in fermentation. *Canarium album* were fermented with different fermentation time (3, 6, 9, 12 days). Effectiveness of fermentation time in *Canarium album* fermentation was based on value of pH to total phenolic compounds (mg/g), total flavonoids (mg/g) and total triterpenoids (mg/g), trolox equivalent antioxidant capacity (mmol/mg). Results were depicted in table 3. It's clearly noticed that 9 days of fermentation was optimal for *Canarium album* fermentation. So we selected this value for application.

Green olives are lactic acid fermented following sodium hydroxide (lye) treatment to remove bitterness (Sanchez et al., 2000). The bacteria involved are *Lb. plantarum, Lb. brevis, P. cerevisiae and Lc. mesenteroides* (Borcakli et al. 1993, Duran-Quintana et al. 1999). The pH varied between 4.0–4.5. The optimum fermentation temperature is 24ºC. The fermentation period usually takes between 2–3 mon. Once the fermentation is complete, the olives are packed in air-tight jars and sterilized, which produces a good quality product with a long storage life. Salting is normally done at 1–10% brine solution (Tsapatsaris and Kotzekidou 2004).

With the aim of formulating a probiotic food, the green olive was used as a vehicle for incorporating probiotic bacterial species such as *Lactobacillus rhamnosus, Lb. paracasei*, *Bifidobacterium bifidum* and *B. longum*. All these strains showed a good survival rate with a recovery of about 106 cfu (colony forming units)/g in olives after 30 d of storage (Lavermicocca et al. 2005).

4. CONCLUSION

Chinese olive fruit extracts contain various phenolic compounds and triterpenoids which showed well antioxidant activities and protective effects against glucose-mediated protein modification. Pickling is one of the ancient ways of food preservation, and it was a possible way of preserving the foods, especially seasonal foods, before the invention of modern preservative machines like the refrigerator. Pickle is the good source of antioxidants, probiotics, vitamins (vitamin C, A, K, and folate), and minerals (iron, calcium, and potassium). Lactic acid fermentation is considered a simple and useful form of biotechnology to enhance the safety, nutritional, sensory and shelf life properties of Chinese olive (*Canarium album*) fruit.

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