



Effectiveness of *Pouzolzia zeylanica*, *Curcuma longa*, *Piper nigrum*, *Capsicum annum* to Stability of Dried Salted Tilapia during Storage

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Abstract.

Tilapia fish protein has a high degree of digestibility and also considered as a rich source of lysine and sulphur containing amino acids. This fish is very rapidly susceptible to spoilage due to various intrinsic characteristics and microbial contamination. Sun drying is one of the traditional method carried out to preserve this fish. However dried fish are heavily infested by blow flies. *Pouzolzia zeylanica* was known to control fly larvae during food processing due to insecticidal activity. The active ingredient of turmeric (*curcuma longa*) having pesticidal action was curcumin. Black pepper (*piper nigrum*), red chilli (*capsicum annum*) are also responsible for the antimicrobial activity. In order to overcome this drawback a study was carried out with some natural spices and herbs (*pouzolzia zeylanica*, *curcuma longa*, *piper nigrum*, *capsicum annum*) not only to prevent the rancidity in dried salted fish but also control it from microbial proliferation. Results from this research revealed that *pouzolzia zeylanica* and *curcuma longa* are ideal in processing and storage of dried salted tilapia. It can be kept well for a period of 12 months. The spices and herbs used for this study were more effective in preserving fish products due to their phytochemical properties, resistance against bacteria and rancidity limitation.

Keywords: *Pouzolzia zeylanica*, *Curcuma longa*, *Piper nigrum*, *Capsicum annum*, dried salted tilapia, rancidity, microbial proliferation

I. INTRODUCTION

Drying is considered as a traditional and widely used method of processing the fish mainly found in the tropical countries. Fishes are very rapidly susceptible to spoilage due to various intrinsic characteristics and microbial contamination from various sources (Venu Gopal et al, 1997). Sun dried fishes are one of the major nutrient providers to the low income people. Salted dried fish are heavily infested by blow flies. Large amount poly unsaturated fatty acids present in the fish lipid makes them highly susceptible to oxidation (Huss, 1995; Boran et al, 2006). This lipid oxidation is a chain reaction which is a continuous supplier of free radicals which further helps to initiate the chain reaction. This lipid oxidation in fish is the main producer of off-odor and off-flavor and reduces the nutritional value.

Pouzolzia zeylanica is considered as a perennial herb. Leaves are ovate or ovate-lanceolate, obtuse, acute or acuminate, entire. Their leaves contains flavone, flavonoids, tannin, carotene, carotenoids, ascorbic, tartaric, malic and pectic acids, gum, minerals and - β -D-glucopyranoside; β -sitosterol-3-O- β their salts; quercetin, vitexin, isovitexin, phylanthin, methyl sterate and sitosterol, daucosterol, oleanolic acid (Fu et al., 2012); leaf powder also contains carbohydrates, gums, reducing sugar, alkaloids, steroids, glycosides, tannins, flavonoids and saponins (Saha and Paul, 2012). Phytochemical derived from plant consisting of phenols and flavonoids possess antioxidant properties, which are useful to scavenge reactive oxygen species (Md Solayman Hossain et al., 2016). Leaves are anthelmintic and vulnerary; used as a cicatrizant for gangrenous ulcers, in syphilis and gonorrhoea.

Leaf juice is used as galactagogic. Poultice of the herb is applied to sores, boils and to relieve stomachache. Steroids (Stigmasterol and β -Sitosterol) and triterpenoid (friedelin) has antitumor or pesticidal activity (Brazendranath Sarkar et al., 2014). The plant *Pouzolzia indica* claimed to be useful in treating snake poison in the Indian system of medicine (Ahmed et al., 2010). *Pouzolzia zeylanica* plant can be used as fresh or dried plant, decoction drunk to treat cough, pulmonary tuberculosis, sore throat, enteritis, dysentery (Chi, 2012). Extracts of *Pouzolzia zeylanica* possessed antibacterial, antifungal and cytotoxic activities (Paul and Saha, 2012; Saha et al., 2012; Saha and Paul, 2012). *Pouzolzia zeylanica* extracts very good antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Dibyajyoti Saha et al., 2012). Therefore, *Pouzolzia zeylanica* was used to control fly larvae during food processing due to insecticidal activity (Nguyen Ngoc Bao Chau et al., 2017). The extraction of phenolic compounds from *Pouzolzia Zeylanica* L. Benn was conducted by using pure water as a solvent. Total polyphenol, flavonoid and tannin content extracted by these optimized conditions were achieved (921 mgGAE/100g dried material (DM), 563 mgQE/100g DM and 643 mgTAE/100g DM, respectively) (Nguyen Duy Tan et al., 2017). A study aimed to optimize additional carrier concentration for spray drying of *Pouzolzia zeylanica* extract (Nguyen Duy Tan and Nguyen Minh Thuy, 2018).

Turmeric (*Curcuma longa*) is an essential spice all over the world with a distinguished human use. Apart from the use as spice, it is used as traditional medicine because of its beneficial properties. Current traditional medicine claims its powder against gastrointestinal diseases, especially for

biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, coryza and cough (Shekhar Kumar Sinoriya et al., 2018). The coloring principle of turmeric is called curcumin, which has yellow color and is the essential component of this plant (Ammon HP et al., 1992). One of the major problems associated with the lengthy sun-drying of fish is the infestation of the products by the blowfly and beetle larvae. To avoid such infestations and microbial contaminations salt and salt-turmeric was used combined in order to achieve the desired product. Being a safe, antimicrobial and incidental food additive, toxic for some microorganisms, depressor of water activity (a_w) of the food, sodium chloride has been used as a seasoning and flavor enhancer as well as a preservative or curing agent (Turan H. et al., 2007; Leroi F. et al., 2000). The active ingredient of turmeric (*Curcuma longa*) having pesticidal action is curcumin.

Black pepper (*Piper nigrum*), sometimes called Indian Long Pepper, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. It is a close relative of the black pepper plant, and has a similar, though generally hotter, taste. The root and fruit of *Piper nigrum* are used in palsy, gout and lumbago. The fruits have a bitter, hot, sharp taste, tonic to the liver, stomachic, emmenagogue, abortifacient, aphrodisiac and digestive (P. Ganesh et al., 2014). They have a pungent pepper-like taste and produce salivation and numbness of the mouth. The fruits and roots are attributed with numerous medicinal uses, and may be used for diseases of respiratory tract, viz., cough, bronchitis, asthma etc; as counter-irritant and analgesic when applied locally for muscular pains and inflammation; as snuff in coma and drowsiness and internally as carminative. Black pepper (*Piper nigrum*) has a well-known antioxidant and radical scavenging properties which have been well documented (Gülcin, 2005). Piperine an alkaloid the major constituent of piper amides present in the skin and seed of the black pepper is responsible for the antimicrobial activity. Extract of black pepper (*Piper nigrum*) has protective or disease preventive properties against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Proteus* sp. (P. Ganesh et al., 2014).

Red chili (*Capsicum annum*) is one of the most consumed vegetables worldwide, after tomato, because of its exquisite flavor and high nutritional value. The genus *Capsicum* represents a potential source of capsaicinoids. *Capsicum* shows the presence of a higher content of sterols and lower content of steroids. There was a higher percentage of citric acid, malic acid and tartaric acid in *Capsicum* tissues without presence of oxalic acid (Luis Germán LópezValdez et al., 2016). Chili powder (*Capsicum annum*) is also known to have antimicrobial properties. *Capsicum annum* consists of the known inhibitory substance capsaicin (Cichewicz and Thorpe., 1996). The flavor and pungent power of these peppers varies widely and so do their contents of capsaicin and its capsaicinoid analogs (Dorantes L, et al. 2000). Many studies confirmed benefits of oregano for human health and its use for the treatment of a vast list of ailments, including respiratory tract disorders such as cough, as an oral antiseptic, in urinary tract

disorders and in various dermatological infections (alleviation of itching, healing crusts, and insect stings), viral infections and even good for cancer.

Some natural spices and herbs not only provide good color and flavor to the product but also contain antioxidants that inhibit lipid peroxidation and other free radical-mediated processes. They not only prevented the rancidity in fish but also controlled it from microbial spoilage (Takao et al., 1994). The objective of the present study was to evaluate effectiveness of different combination of herbs (*Pouzolzia zeylanica*, *Curcuma longa*, *Piper nigrum*, *Capsicum annum*) mixture along with salt in controlling rancidity as well as microbial growth in of dried salted tilapia fillet.

II. MATERIALS AND METHOD

2.1 Materials

We collected different spices and herbs such as *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* from Nga Nam district, Soc Trang province, Vietnam. After collecting, they were stored at a temperature of 8°C and conveyed to laboratory within 8 hours for experiments. They were washed thoroughly under tap water to remove dirt, dust and adhered unwanted material. After that they were dried to 12% of moisture content and then grinded into powder ready to use. Tilapia fishes were collected from Chau Thanh district, Soc Trang province, Vietnam. They were raised following VietGap without using antibiotic to ensure food safety. After harvesting, they were kept in ice chest and move to laboratory within 4 hours to fillet. Beside herbs and tilapia fishes we also used other materials during the research such as NaCl, sugar, monosodium glutamate, garlic. Lab utensils and equipments included digital weight balance, dry oven.



Figure 1. *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum*

2.2 Methods

2.2.1 Primary analysis of chemical composition (total polyphenol content, flavonoid, tannin) in different spices and herbs

Chemical composition (total polyphenol content, flavonoid, and tannin) in different spices and herbs was examined. Total polyphenol content (mg GAE/100 g) was determined by FolinCiocalteu reagent method (Hossain et al., 2013). Aluminum chloride colorimetric method was used for flavonoids (mg QE/100 g) determination (Eswari et al., 2013; Mandal et al., 2013). Tannin content (mg TAE/100

g) was determined by Folin-Denis method (Laitonjam et al., 2013).

2.2.2 Effect of herb powder supplementation to chemical and microbial characteristics of dried salted tilapia fillet

Tilapia fillet was prepared by mixing NaCl (0.5%), sugar (0.2%), monosodium glutamate (0.01%, garlic (0.1%). Then tilapia fillet would be treated with 2% of different herb powder such as *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* before drying at 40°C in 20h. The optimal herb was selected by analyzed results of peroxide value (mEqO2/ kg), thiobarbituric acid (mg malonaldehyde/ kg), *coliform* (cfu/g) in the dried salted tilapia fillet. Periodically sampling was conducted during storage (0, 3, 6, 9 and 12 months) on the quality of the dried salted tilapia fillet.

2.3 Chemical and microbial evaluation on dried salted tilapia fillet

Peroxide value (mEqO2/ kg) was determined using the CDR FoodLab® instrument. Thiobarbituric acid (mg malonaldehyde/ kg) was measured by 1,1,3,3-tetraethoxypropane (Torres-Arreola et al., 2007). *Coliform* (cfu/g) was measured by 3M-Petrifilm.

2.4 Statistical analysis

The Methods were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan’s multiple range test (DMRT). Statistical analysis was performed by the Statgraphics Centurion XVII.

III. RESULTS & DISCUSSION

3.1 Primary analysis of chemical composition (total polyphenol content, flavonoid, tannin) in different spices and herbs

Table 1. Total polyphenol content (mg GAE/100 g), flavonoid (mg QE/100 g), tannin (mg TAE/100 g) in *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* powder

Spices and herbs	<i>Pouzolzia zeylanica</i>	<i>Curcuma longa</i>	<i>Piper nigrum</i>	<i>Capsicum annum</i>
Total polyphenol content (mg GAE/100 g)	919.02±0.03a	678.76±0.01b	544.19±0.02c	487.94±0.02d
Flavonoid (mg QE/100 g)	568.31±0.02a	495.43±0.03b	423.28±0.02c	374.25±0.01d
Tannin (mg TAE/100 g)	647.20±0.01a	579.27±0.04b	454.38±0.01c	388.29±0.03d

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. Peroxide value (mEqO2/ kg) in the dried salted tilapia fillet by *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* powder during preservation

Preservation (months)	Control	<i>Pouzolzia zeylanica</i>	<i>Curcuma longa</i>	<i>Piper nigrum</i>	<i>Capsicum annum</i>
0	0	0	0	0	0
3	0.39±0.01a	0.13±0.00d	0.15±0.03c	0.18±0.00b	0.17±0.00bc
6	0.75±0.02a	0.15±0.00d	0.19±0.01c	0.22±0.01b	0.21±0.00bc
9	0.84±0.00a	0.17±0.01d	0.21±0.02c	0.25±0.03b	0.23±0.02bc
12	0.98±0.02a	0.20±0.02d	0.24±0.03c	0.28±0.02b	0.26±0.01bc

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. Thiobarbituric acid (mg malonaldehyde/ kg) in the dried salted tilapia fillet by *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* powder during preservation

Main composition (total polyphenol content, flavonoid, tannin) in different spices and herbs) in *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* powder was confirmed (table 1). Results showed that these spices and herbs had high antioxidant capacity suitable for fish preservation.

These results were similar to findings by Tanzeela Nisar et al., (2015), Nguyen Duy Tan et al., (2017), Deepshikha Gupta (2013), Gisele Teixeira Souza Sora et al., (2015). The spices and herbs used to prepare the sample had high active phenolic antioxidant property which inhibited the free-radical mediated damages like lipid oxidation thus preventing oxidative rancidity (Khatun et al. 2006). This helped to increase protection against insects, pests, bacteria, fungus and other pathogens acting as a natural preservative and a taste enhancing ingredient.

3.2 Effect of herb powder supplementation to chemical and microbial characteristics of dried salted tilapia fillet during storage

Tilapia fillet was prepared by mixing NaCl (0.5%), sugar (0.2%), monosodium glutamate (0.01%, garlic (0.1%). Then tilapia fillet would be treated with 2% of different herb powder such as *Pouzolzia zeylanica*, *Curcuma longa*, *Piper nigrum*, *Capsicum annum* before drying at 40°C in 20h. Analyzed results of peroxide value (mEqO2/ kg), thiobarbituric acid (mg malonaldehyde/ kg), *coliform* (cfu/g) in the dried salted tilapia fillet showed that *Pouzolzia zeylanica* was the best one for dried salted tilapia preservation. There was a steady decrease in bacterial count during the storage period in all the four treatments. Meanwhile bacteria increased in control samples.

Preservation (months)	Control	<i>Pouzolzia zeylanica</i>	<i>Curcuma longa</i>	<i>Piper nigrum</i>	<i>Capsicum annum</i>
0	0	0	0	0	0
3	4.49±0.02a	3.24±0.03d	3.38±0.00c	3.55±0.02b	3.41±0.03bc
6	5.68±0.03a	3.29±0.01d	3.43±0.01c	3.68±0.02b	3.57±0.00bc
9	6.40±0.00a	3.35±0.00d	3.50±0.02c	3.74±0.01b	3.62±0.02bc
12	7.26±0.01a	3.41±0.00d	3.57±0.00c	3.92±0.03b	3.87±0.01bc

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

Table 4. Coliform (cfu/g) in the dried salted tilapia fillet by *Pouzolzia zeylanica*; *Curcuma longa*, *Piper nigrum*, *Capsicum annum* powder during preservation

Preservation (months)	Control	<i>Pouzolzia zeylanica</i>	<i>Curcuma longa</i>	<i>Piper nigrum</i>	<i>Capsicum annum</i>
0	1.1x10 ² ±0.02a	1.1x10 ² ±0.02a	1.1x10 ² ±0.02a	1.1x10 ² ±0.02a	1.1x10 ² ±0.02a
3	1.4x10 ² ±0.01a	5.2x10 ¹ ±0.01d	6.4x10 ¹ ±0.01c	7.4x10 ¹ ±0.02b	7.3x10 ¹ ±0.03bc
6	2.0x10 ² ±0.02a	4.3x10 ¹ ±0.02d	4.9x10 ¹ ±0.02c	6.4x10 ¹ ±0.03b	5.3x10 ¹ ±0.00bc
9	2.5x10 ² ±0.03a	2.2x10 ¹ ±0.03d	2.3x10 ¹ ±0.03c	5.3x10 ¹ ±0.01b	4.8x10 ¹ ±0.01bc
12	2.8x10 ² ±0.01a	0.6x10 ¹ ±0.00d	0.7x10 ¹ ±0.00c	2.5x10 ¹ ±0.00b	2.0x10 ¹ ±0.00bc

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

Drying when combined with both salt and spices herb combination had dual effects which result in lowering of the water activity (a_w) level and a specific inhibitory effect on the growth of some species of microorganisms. The main cause of decrease in moisture was due to osmotic migration of salt and spices and herb used in had much more capacity to migrate inside the fish and which result in oozing water out of the fish. This decrease in moisture leads to increase in spice content and consequently extend shelf life of the product. The dried fish sample generally contains more nutrients than fresh fish. In storage condition, normally the protein content starts to decrease significantly with the time because water soluble protein diffused out to the surrounding for exosmosis (Hassan, M.N. et al., 2013) whereas here the spices and herb treatment used in sample were very effective in preventing the degradation of protein. The combination of spices and herb treatment with solar drying in sample resulted in the decrease of microbial levels. According to (Abhishek S. 2011) spices and herbs have a significant antimicrobial effect, against various microorganisms and impart various nutritive health benefits to the body. Whereas in case of control sample due to the absence of spices and herbs lead to lipid oxidation in the fish and resulted in the rancidity and off flavor.

IV. CONCLUSION

Pouzolzia zeylanica, *Curcuma longa*, *Piper nigrum*, *Capsicum annum* are native plant and have been demonstrated their applicability as medical plant. Among them, *Pouzolzia zeylanica* and *Curcuma longa* had the highest chemical preservative and antioxidant properties. They were two ideal herbs for fish storage. Sun drying processed to achieve a reasonable shelf life of dried salted fish at ambient tropical temperature. This approach created an effective strategy to provide a simple, cheaper, healthier, tastier and safer method of fish preservation in developing countries like Vietnam. Salting method is used to remove

water from fish body and makes the fish to dry whereas spices and herbs helped to remove water from fish body and also favorably adds some nutrients and bioactive compounds which prevented the growth of microorganisms. So, this way can be recommended as a preservation technique in large scale dry fish production

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