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Production of Pickled Fermented Snakehead fish (*Channa punctatus)*

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Abstract.
Snakehead fish (*Channa punctatus*) is one of the favourite fresh water fish in Vietnam. It is a very important food due to high protein content and nutritional value. Snakehead fish has a high potential to be used as a promising acceptable source of medicines and nutrients for the treatment of serious diseases as well as for the improvement of general body tones of human beings to a greater extent. Honey is a natural sweet product, made by the honey bees using their own secretion deposit and dehydrates with various nectar of flower and excretion of insect on the plants part, then transform and stored it in the honey combs and ripened into honey. Fermentation is considered as a common approach in food storage; it contributes an essential duty in improvement of nutritional and functional characteristic of foodstuff. Honey-pickled snakehead fish has good source of peptides and amino acids. Therefore, objective of this investigation focused on different technical aspects such as the effect of Salt, roasted rice powder, sugar and alcohol concentration used for primary treatment; honey ratio mixing with snakehead fish, fermentation temperature and time influencing to the amino acid content, pH, water activity and sensory characteristics honey-pickled snakehead fish. Results revealed that 16% of salt, 1.6% of roasted rice powder, 16% of sugar, 9% of alcohol addition together with 16% honey in snakehead fish mixture; fermentation at 28^{o}C for 9 months, the final honey-pickled snakehead fish had the good a pleasant taste and appearance. Pickle fermented snakehead fish has the potential of becoming ready-to-eat, self stable, highly acceptable health food products of indigenous origin. This delicious pickled snakehead fish was highly welcomed by customers.
Keywords: *Snakehead fish, honey, salt, sugar, alcohol, pickle*

I. INTRODUCTION

Snakehead fish is an obligatory air-breather and predaceous fish that resides in swamps, slow-flowing streams and in crevices near riverbanks. It is consumed for its dietary proteins and a remedy for healing of wound (Lay-Harn Gam et al., 2005). The fish is carnivorous and consumes fish, frog, snakes, insects, earthworms and tadpoles, therefore its flesh claimed to be rejuvenating, particularly for those recuperating from a serious illness (M Aminur Rahman et al., 2012). Snakehead fish is largely used for food, traditional medicines and pharmacological therapeutics including anti-microbial, anti-inflammatory, cell proliferation, induction of platelet accretion and anti-nociceptive activities. Snakehead extract contains high levels of essential amino acids and a good profile of fatty acids that could directly improve tissue growth, wound healing, nutraceutical supplements and pharmaceutical products. From the view point of food sources, they are playing as the role of functional foods, which provide health benefit beyond basic nutrition (MohdShafri MA & Abdul Manan MJ, 2012). It contains essential fatty acids, indicating the abundant presence of 30% C16:0 along with other major fatty acids of C20:4 (19%), C18:0 (15%), C22:6 (15%) and C18:1 (12%). It also contains 19.0% of arachidonic acid (C20:4), a precursor for prostaglandin and thromboxane biosynthesis, which represents the best composite for wound healing processes (Tan, B. H. and Azhar, M. E., 2014). Individually in fillet and mucus extracts of snakehead is found to exhibit a concentration dependent antinociceptive activity. Recently snakehead fish has been used as biomedical and nutraceutical products for clinical trials, treatment of several chronic diseases as well as improvement of human health and therapeutics to a greater extent (Rahman MA et al., 2018).

Honey is a very complex natural product that contains sugars, organic acids, amino acids, proteins, minerals, vitamins, lipids, aromatic compounds, flavonoids, pigments, waxes, pollen grains, enzymes and other phytochemicals (Gomes et al. 2010; Almeida-Muradin et al. 2013). Honey has distinct and unique flavor, aroma, color and composition that depend on a lot of variables: nectar composition of the flora source, bee species, climate, environmental and seasonal conditions, agricultural practices, geographical origin, and techniques used during honey extraction and storage (Castro et al. 2010; Almeida-Muradin et al. 2013). Honeys may be classified as mono-floral or poly-floral depending on whether a dominating pollen grain, originated from only one particular plant (mono-floral honey) or no dominant pollen type in the sample is found (poly-floral honey) (Moussa et al. 2012). Beekeeping (honey production) is also considered a natural resource-conserving and environmentally friendly activity (Abera Hailu Degaga, 2017). Fermented products from Snakehead fish (*Channa punctatus*) such as fermented snakehead fish paste, pickled snakehead fish are widely consumed in Vietnam. Regarding to production of pickled snakehead fish, there were serveral outstanding researches. Thai fermented fish product was prepared from snakehead fish, salt, palm syrup and sometimes roasted rice (Paludan-Müller C et al., 2002). Quality and storage stability of low acid Murrel (*Channa striatus*) fish pickle at room temperature was examined **(**Sahu, B. B et al. 2012**).** The lack of standardization of the processing methods and hygiene during processing, with their detrimental effects on the quality and the safety of the endproducts are the major problems to overcome to ensure the promotion of fermented fish products sector (Anihouvi V.B. et al., 2012). Therefore, objective of this research focused on different technical aspects such as the effect of Salt, roasted rice powder, sugar and alcohol concentration used for primary treatment; honey ratio mixing with snakehead fish, fermentation temperature and time influencing to the amino acid content, pH, water activity and sensory characteristics honey-pickled snakehead fish.

II. MATERIALS AND METHOD

2.1 Material

We collected snakehead fish (*Channa punctatus)* from Ca mau province, Vietnam. They must be reared following VietGAP to ensure food safety. After collecting, they must be temporarily preserved by flake ice and conveyed to laboratory within 8 hours for experiments. They were washed and sanitized under washing tank having 25 ppm chlorine with a support of air bubble blowing to remove foreign matters. Besides *Channa punctatus* we also used another material during the research such as chlorine, Salt, roasted rice powder, sugar, alcohol, honey, ceramic jar. Lab utensils and equipments included digital weight balance, water activity meter, pH meter, amino acid analyzer.

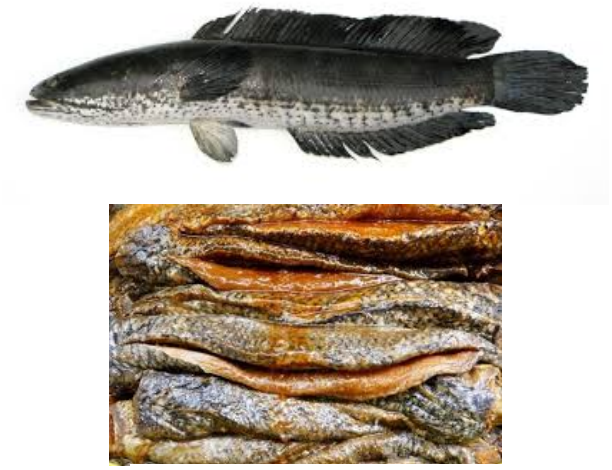

Figure 1. Snakehead fish (*Channa punctatus*) and its pickled product

2.2 Researching procedure

2.2.1 Effect of Salt, roasted rice powder, sugar, alcohol concentration to amino acid, pH, water activity and sensory score of the honey-pickled snakehead fish (Channa punctatus)

Snakehead fishs (*Channa punctatus*) were treated with different ratios: Salt (4%, 8%, 12%, 16% 20%), roasted rice powder (1.0%, 1.2%, 1.4%, 1.6%, 1.8%), sugar (4%, 8%, 12%, 16%, 20%), alcohol (3%, 5%, 7%, 9%, 11%), honey (2%, 4%, 6%, 8%, 10%) to create a pleasant taste and appearance of honey-pickled Snakehead fish. All samples fermented at 30^{o}C in 6 months. Five honey-pickled snakehead fishs were chosen randomly to analyse amino acid (g/100g), pH, water activity (a_w), and sensory score.

2.2.2 Effect of fermentation temperature and time to amino acid, pH, water activity and sensory score of the honey-pickled snakehead fish (Channa punctatus)

Snakehead fishs (*Channa punctatus*) were treated with different ratios: Salt (4%, 8%, 12%, 16% 20%), roasted rice powder (1%, 1.2%, 1.4%, 1.6%, 1.8%), sugar (%, 8%, 12%, 16%, 20%), alcohol (3%, 5%, 7%, 9%, 11%), honey (2%, 4%, 6%, 8%, 10%) to create a pleasant taste and appearance of honey-pickled snakehead fish. All samples fermented different temperature and time (26^{o}C in 12 months, 28^{o}C in 9 months, 30^{o}C in 6 months, 32^{o}C in 3 months). Five honey-pickled snakehead fishs were chosen randomly to analyse amino acid (g/100g), pH, water activity (a_w), and sensory score.

2.2.3 Shelf-life of the honey-pickled snakehead fish (Channa punctatus) during storage

Honey-pickled snakehead fish *(Channa punctatus)* products were kept in ambient temperature condition (28±2^{o}C). Amino acid (g/ 100g), pH, water activity (a_w), sensory score were used as quality indicators by sampling in 3 months interval for 12 months.

2.3 Physico-chemical, microbial and sensory analysis

Amino acid was measured by using an amino acid analyzer. pH of samples was measured using a pH meter. Water activity was assessed by using a water activity meter. Sensory score of fermented snakehead fish (*Channa punctatus)* paste was assessed by a group of panelist using the 9-point hedonic scale.

2.4 Statistical analysis

The experiments 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 XVI.

III. RESULT & DISCUSSION

3.1 Effect of Salt, roasted rice powder, sugar, alcohol, honey ratio supplemented to snakehead fish mixture to amino acid, pH, water activity and sensory score of the honey-pickled snakehead fish *(Channa punctatus)*

Snakehead fishs (*Channa punctatus*) were treated with different ratios: Salt (4%, 8%, 12%, 16% 20%), roasted rice powder (1.0%, 1.2%, 1.4%, 1.6%, 1.8%), sugar (4%, 8%, 12%, 16% 20%), alcohol (3%, 5%, 7%, 9%, 11%), honey (2%, 4%, 6%, 8%, 10%) to create a pleasant taste and appearance of honey-pickled Snakehead fish. All samples fermented at 30^{o}C in 6 months. Five honey-pickled Snakehead fishs were chosen randomly to analyse amino acid (g/100g), pH, water activity (a_w), and sensory score. From table 1, 2, 3, 4, 5; the best quality of honey-pickled snakehead fish (*Channa punctatus*) would be achieved by 16% of salt, 1.6% of roasted rice powder, 16% of sugar, 9% of alcohol addition together with 8% honey in snakehead fish mixture

Table 1. Effect of salt concentration to amino acid, pH, water activity and sensory score of the honey-pickled snakehead fish *(Channa punctatus)*

Salt	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
4%	38.42±0.02^b	5.80±0.02^a	0.65±0.02^a	5.14±0.02^c
8%	39.11±0.00^{ab}	5.77±0.01^{ab}	0.60±0.00^{ab}	6.29±0.01^b
12%	40.04±0.01^{ab}	5.75±0.01^{bc}	0.53±0.01^b	6.34±0.02^{ab}
16%	40.85±0.03^a	5.73±0.01^c	0.48±0.00^{bc}	6.42±0.01^a
20%	40.90±0.01^a	5.76±0.03^b	0.42±0.03^c	5.89±0.01^{bc}

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. Effect of roasted rice powder concentration to amino acid, pH, water activity and sensory score of the honey-pickled snakehead fish *(Channa punctatus)*

Roasted rice powder	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
1.0%	40.85±0.03^{b}	5.73±0.01^{b}	0.48±0.00^{a}	6.42±0.01^{c}
1.2%	40.85±0.01^{b}	5.73±0.01^{b}	0.48±0.01^{a}	6.53±0.02^{bc}
1.4%	40.86±0.00^{b}	5.73±0.01^{b}	0.49±0.02^{a}	6.79±0.01^{b}
1.6%	**40.87±0.01^{ab}**	**5.74±0.03^{ab}**	**0.49±0.01^{a}**	**6.94±0.03^{a}**
1.8%	40.89±0.02^{a}	5.77±0.02^{a}	0.49±0.01^{a}	6.80±0.01^{b}

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. Effect of sugar concentration to amino acid, pH, water activity and sensory score of the honey-pickled Snakehead fish *(Channa punctatus)*

Sugar	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
4%	40.87±0.01^{c}	5.74±0.03^{a}	0.49±0.01^{a}	6.94±0.03^{c}
8%	40.91±0.01^{bc}	5.70±0.02^{b}	0.43±0.02^{ab}	7.11±0.01^{b}
12%	41.04±0.01^{ab}	5.68±0.03^{bc}	0.40±0.01^{b}	7.28±0.02^{ab}
16%	**41.05±0.02^{a}**	**5.65±0.00^{c}**	**0.36±0.01^{bc}**	**7.86±0.01^{a}**
20%	41.01±0.01^{b}	5.72±0.01^{ab}	0.32±0.03^{c}	7.30±0.01^{ab}

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 4. Effect of alcohol concentration to amino acid, pH, water activity and sensory score of the honey-pickled Snakehead fish *(Channa punctatus)*

Alcohol concentration	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
3%	41.05±0.02^{c}	5.65±0.00^{a}	0.36±0.01^{a}	7.86±0.01^{c}
5%	41.13±0.01^{bc}	5.64±0.02^{ab}	0.36±0.01^{a}	7.94±0.01^{bc}
7%	41.18±0.01^{ab}	5.64±0.01^{ab}	0.36±0.01^{a}	8.01±0.01^{b}
9%	**41.23±0.01^{a}**	**5.63±0.03^{b}**	**0.36±0.02^{a}**	**8.18±0.02^{a}**
11%	41.15±0.01^{b}	5.65±0.01^{a}	0.36±0.01^{a}	8.10±0.01^{ab}

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 5. Effect of honey ratio supplemented to snakehead fish mixture to amino acid, pH, water activity and sensory score of the honey-pickled Snakehead fish *(Channa punctatus)*

Honey ratio	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
4%	41.23±0.01^{c}	5.63±0.03^{a}	0.36±0.02^{a}	8.18±0.02^{c}
8%	41.30±0.02^{bc}	5.60±0.01^{ab}	0.34±0.00^{ab}	8.23±0.02^{bc}
12%	41.37±0.00^{ab}	5.58±0.02^{bc}	0.32±0.00^{b}	8.29±0.02^{ab}
16%	**41.44±0.01^{a}**	**5.55±0.01^{c}**	**0.29±0.01^{bc}**	**8.38±0.01^{a}**
20%	41.32±0.03^{b}	5.59±0.02^{b}	0.25±0.02^{c}	8.25±0.01^{b}

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 6. Effect of fermentation temperature and time to amino acid, pH, water activity and sensory score of the honey-pickled snakehead fish *(Channa punctatus)*

Fermentation	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
26^{o}C : 12 months	41.32±0.03^{b}	5.51±0.01^{b}	0.29±0.01^{a}	8.29±0.02^{b}
28^{o}C : 9 months	**42.58±0.01^{a}**	**5.52±0.02^{b}**	**0.29±0.00^{a}**	**8.47±0.01^{a}**
30^{o}C : 6 months	41.44±0.01^{ab}	5.55±0.01^{ab}	0.29±0.01^{a}	8.38±0.01^{ab}
32^{o}C : 3 months	40.03±0.02^{c}	5.61±0.03^{a}	0.29±0.00^{a}	7.44±0.03^{c}

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 7. Shelf-life of the honey-pickled snakehead fish *(Channa punctatus)* during storage

Preservation (months)	Amino acid (g/100g)	pH	Water activity (a_w)	Sensory score
0	37.03±0.02^{e}	4.60±0.02^{a}	0.29±0.02^{a}	8.24±0.01^{ab}
3	40.12±0.02^{d}	4.55±0.01^{ab}	0.29±0.01^{a}	8.30±0.02^{a}
6	41.48±0.01^{c}	5.54±0.01^{ab}	0.29±0.01^{a}	8.36±0.01^{ab}
9	**42.58±0.01^{b}**	**5.52±0.02^{b}**	**0.29±0.00^{a}**	**8.47±0.01^{a}**
12	43.79±0.02^{a}	5.52±0.02^{b}	0.29±0.02^{a}	8.50±0.02^{c}

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%).

Thai fermented fish product was prepared from snakehead fish, salt, palm syrup and sometimes roasted rice. They studied the effects of different salt concentrations on decrease in pH and on microflora composition during fermentation. Two low-salt batches were prepared, containing 6% and 7% salt (w/w) as well as two high-salt batches, containing 9% and 11% salt. pH decreased rapidly from 6 to 4.5 in low-salt batches, whereas in high-salt

batches, a slow or no decrease in pH was found (Paludan-Müller C et al., 2002). Quality and storage stability of low acid Murrel (*Channa striatus*) fish pickle at room temperature was examined. Low acid murrel meat pickle was prepared using deboned meat from marketable size murrel (700 + 59 g). The product was evaluated after an elapse of seven days on maturation for change in physico-chemical, microbiological and organoleptic properties at an interval of 15 days up to 60 days at 32 + 0.50C. Results showed that pH and titratable acidity of the low acid pickle was 4.83 and 0.68, where as in control murrel pickle, these values were 4.68 and 0.74 respectively. After 60 days of storage period microbiological count and sensory quality traits did not show appreciable change and remained satisfactory throughout the storage period. Low acid pickles had signifcantly lower sourness and high overall acceptability compared to the control **(**Sahu, B. B et al. 2012**).** Effects of salt, sugar and starter culture on fermentation and sensory properties in *Shidal* (a fermented fish product) was verified (Armaan Ullah Muzaddadi and Prasanta Mahanta, 2012). Rainbow Sardines and mackerel were pickled according to this cure, salt ratio adopted being 1:3 by weight of fish (Suryanarayana Rao S. V. et al., 1958). Salt concentration 1.0-3.5% and acetic acid 1- 2%, the stability of pickled fish was low (Kreuzer, R. 1990). The firmness of marinated prawns (*Machrobrachium rosenbergii*) in various Salt and acidity was evaluated. Nowadays people prepared seafood pickle by using organic solution with Salt and spices (Saritha, K. et al., 2014).

3.2 Effect of fermentation temperature and time to amino acid, pH, water activity and sensory score of the honey-pickled Snakehead fish *(Channa punctatus)*

Snakehead fishs (*Channa punctatus*) were treated with different ratios: Salt (4%, 8%, 12%, 16% 20%), roasted rice powder (1.0%, 1.2%, 1.4%, 1.6%, 1.8%), sugar (4%, 8%, 12%, 16% 20%), alcohol (3%, 5%, 7%, 9%, 11%), honey (4%, 8%, 12%, 16% 20%) to create a pleasant taste and appearance of honey-pickled Snakehead fish. All samples fermented different temperature and time (26^{o}C in 12 months, 28^{o}C in 9 months, 30^{o}C in 6 months, 32^{o}C in 3 months). Five honey-pickled snakehead fishs were chosen randomly to analyse amino acid (g/100g), pH, water activity (a_w), and sensory score. From table 6, the fermentation process should be conducted at 28^{o}C for 9 months to get a pleasant taste of honey-pickled snakehead fish *(Channa punctatus)*

Fermentation was one of the preservative approaches to maintain the stability of the seafood storage for long time (Jamila Patterson, P.; and Ayyakannu, K., 1997). Shrimp paste was produced by mixing with salt to ferment at ambient temperature (Ernestina, M. et al., 2005). The effect of varying salt concentration (10%, 17.5% and 25%), fermentation temperature (30-35 ^{o}C and 40^{o}C) and fermentation period (3 and 7 days) on microbial, chemical and biochemical changes of tuna viscera during fermentation were investigated (Jesebel R. Besas and Erlinda I. Dizon, 2014).

3.3 Shelf-life of the honey-pickled snakehead fish *(Channa punctatus)* during storage

Honey-pickled snakehead fish *(Channa punctatus)* products were kept in ambient temperature condition (28±2^{o}C). Amino acid (g/ 100g), pH, water activity (a_w), sensory score were used as quality indicators by sampling in 3 months interval for 12 months. From table 7, quality of honey-pickled Snakehead fish *(Channa punctatus)* was nearly stable at ambient temperature (28±2^{o}C) during 12 months of storage.

During pickling process, a large quantity of specific amino acid was produced. Seafood pickle was safe without any toxic bacteria and maintaining durable stability over 6 months at room temperature (Chandrashekhar, T. C, 1979). The preparation of pasteurized marinated shrimp in green curry paste was safe for human consumption until the end of storage period for 15 days at 0-3°C (Jawahar, A.T. et al., 1994). Quality characteristics of fermented sour fish cake (Nham-Pla) were observed. Results indicated that the range of pH readings among a variety of samples varied from 4.50 to 5.80, lactic acid contents varied from 0.49 to 1.13% (w/w), moisture contents varied from 75.7 to 80.1% (w/w), protein contents varied from 14.08 to 14.92% (vv/w), fat content varied from 0.49 to 1.95% (w/w) and salt contents varied from 1.61 to 1.97% (w/w). (M. Sangjindavong et al., 2000).

IV. Conclusion

Snakehead fish is fresh water species and considered as a source of high quality protein and traditional remedy of sickness. Snakehead is not only the healthy diet to eat and relish but are often used as medicine for various diseases. It is a good source of medicinal food because it contains high level of amino acids and fatty acids. Amino acids might have contributed to its pharmacological properties. Pickling is one of the easy and safe means of snakehead fish preservation. Pickled snakehead fishs create essential peptids and amino acids through autolytic and fermented reaction during the fermentation. However there was not many research mentioned to production of pickle snakehead fish (*Channa punctatus*). The main production of this product is salt adding, fermenting, and curing. We have successfully examined different factors affecting to production of honey-pickled Snakehead fish *(Channa punctatus)*. By preserving under ambient temperature (28±2^{o}C), the honey-picked snakehead fish could be maintained shelf-life for 12 months without any preservative. This simple technology can be easily approached to process in household scale.

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