

# EXPLOITATION OF BIOACTIVE COMPONENTS FROM EXTRACTS OF GINGER, GALANGAL TO PRESERVE SQUID AND CUTTLEFISH

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## ABSTRACT

Galangal and Ginger have long been known not only as familiar spices, but also as materials containing many good biological activity components as antibacterial and antioxidant. Raw seafood is materials rapidly spoiled after catching, especially with discoloration or spoilage of squid and cuttlefish. Exploiting natural components from ginger, galangal to preserve squid and cuttlefish and to extend shelf life, to ensure quality and replace the unsafe additive direction is necessary in Viet Nam.

Methods of extracting the bioactive components from ginger, galangal by solvent of ethanol/water and water were selected. The results found that these extracts have a high polyphenol content (from 6.09 to 18.38 mg/g dry powder), as well as high antibacterial ability with some human pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Vibrio parahaemolyticus*, etc. These extracts had high antioxidant ability, which was determined by the DPPH (2,2 - diphenyl -1-picrylhydrazyl) free radical scavenging ability.

Raw squid and cuttlefish preserved by these extracts separately and in combination after catching at 0-4°C conditions, can be stored up to 11-14 days but the qualities still remain good (more than 8-11 days compared with control samples).

**Keywords:** Galangal, ginger, polyphenol, antioxidant, antibacterial, preservation, squid and cuttlefish.

## 1. INTRODUCTION

Raw seafood including squid and cuttlefish are always rapidly spoiled after fishing (Antonio V. Sykes., 2007). In Vietnam, because fishing vessels at the sea are quite rudimental so there is always lack of properly cold, freezing storage prepared to keep the quality of the seafood. So many fishermen want to reduce the declining quality of raw squid and cuttlefish (spoilage,

changing color...) by having used unregulated chemicals for preservation.

Spices like ginger, galangal are always used as marinated spices for seafood materials during processing in Vietnam for making flavor and freshening the seafood. Useful components from ginger, galangal have always been mentioned in many studies as having good antibacterial and antioxidant ability (AO Morakinyo, at al., 2011) and (Shirin Adel P. R, et al., 2011). Therefore, the research team wanted to exploit effectively components from ginger, galangal from Vietnam to preserve fresh squid and cuttlefish, to help extend their shelf life and to ensure food safety for consumers.

## 2. EXPERIMENT

### 2.1 Experimental Apparatus

- There are two varieties of ginger: one has small rhizomes (Re ginger) purchased from Ky Son mountains, Nghe An province and the other has big rhizomes (Trau ginger) purchased from the northern provinces of Vietnam. One variety of galangal purchased from Bac Giang region.

- Raw cuttlefish, squid caught at night and early morning near the coast of Thanh Hoa province (Central Viet Nam), were put in the heat-insulated box fully covered with ice and transported to the laboratory (3-4 hours by car). Then it was processed with extracts of ginger, galangal to study the preservation process.

- Bair Packer environment (Isolation and enumeration of *S. aureus*).

- VRBGA environment (Isolation and enumeration of *Enterobacteriaceae*).

- PCA environment (Isolation and enumeration of total aerobic microorganisms).

### 2.2 Technique

- Isolation and enumeration of *Staphylococcus aureus* according to the TCVN 4830-1: 2005.
- Isolation and enumeration of *Enterobacteriaceae* according to the international standard method F18-1 (UK, 2005).
- Isolation and enumeration of total aerobic microorganisms according to the TCVN 7928: 2008.
- Determination of pH according to the TCVN 4835: 2002 (ISO 2917: 1999).
- Determination of  $\text{NH}_3$  according to the TCVN 3706: 1990.
- Evaluating antibacterial ability of ginger, galangal extracts using dish agar diffusion method.
- Total Phenolics content was determined spectrophotometrically using the modified Folin-Cioalteau colorimetric method (Yasser F.M. Kishk, et al., 2010)
- Evaluating antioxidant activity by free radical scavenging ability with the DPPH assay (Tepe. B, et al., 2005).
- Preparation of Ginger and Galangal extracts: purchased fresh rhizomes of ginger, galangal were washed, then sliced and dried at  $45^\circ\text{C}$  for 20-24 hours, until dry. Cooled and milled to powder and cryopreserved before extracting. Conducted extraction by using the ethanol/water solvent, for ginger selected the ethanol/water solvent of 50% (v/v) and for galangal the solvent of 60% (v/v), extracted at the temperature of  $50^\circ\text{C}$ . And extracted with water solvent at the temperature of  $60^\circ\text{C}$  (the concentration ranges of ethanol/water solvent and temperature of water were studied and published in another article and showed the above parameters were the best parameters). The extraction rate of ginger (galangal) powder/solvent is 1/40. The recovered extracts were concentrated for detaching ethanol and to norm the volume to the same volume at the ratio 1/40 (weight of powder/volume of norm) and stored at the  $-20^\circ\text{C}$ . To evaluate antibacterial, anti-oxidant ability and the ability of preservation of these extracts.

- Notations of extracts are as follows:

Powder of Re/ Trau ginger extracting with ethanol/water 50% (v/v): RGPEW/TGPEW

Powder of Re/ Trau ginger extracting with water at  $60^\circ\text{C}$ : RGPW/TGPW

Galangal powder extracted with ethanol/water 50% (v/v): GPEW/GPEW

Galangal powder extracted with water at  $60^\circ\text{C}$ : GPW/GPW

- Experimental method to preserve squid:

Squid after catching were preserved with ice in heat-insulated box and transported to the laboratory, were soaked for 30 minutes in the extracts of ginger, galangal separately and combined, and keep the temperature of soaking at  $4^\circ\text{C}$ , then scoop out and placed squid and cuttlefish in plastic boxes, placed thick ice layer at the bottom of the box, then the layer of squid, then the layer of ice, to pay attention that the squid should not contact directly with the ice but through one

thin layer of PE. Then keep the boxes in a refrigerator at the temperature of  $0-2^\circ\text{C}$ . Then keep evaluating the physicochemical, microbiological and sensory indicators of the squid sample processed with extracts of ginger, galangal and the control samples which were not processed according to preservation time of 12-13 days.

### 3. ANALYSIS

#### 3.1. Evaluation of biological properties of extracts of ginger, galangal

##### 3.1.1. Determination of the phenolics content of extracts of ginger, galangal

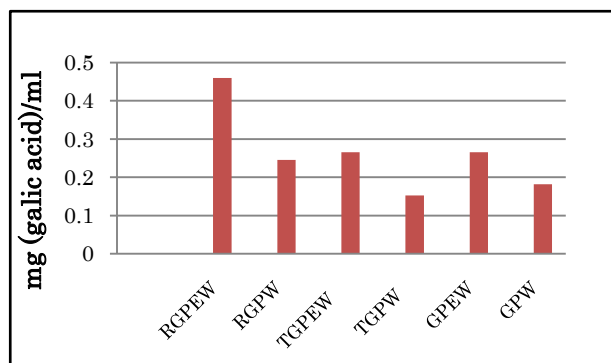


Fig. 1 Phenolics content of extracts of ginger and galangal

The results of Fig 1 showed the two varieties of ginger and 1 variety of galangal currently widely planted in Viet Nam when extracted with the ethanol/water solvent and water solvent giving quite similar phenolics content, except for the RGPEW sample (Re Ginger Powder Ethanol/Water 50/50% (v/v) giving the highest phenolic content, much higher than remaining samples (0.4595 mg/ml or 18.38 mg/g dry powder)

##### 3.1.2. Evaluation of the antioxidant ability

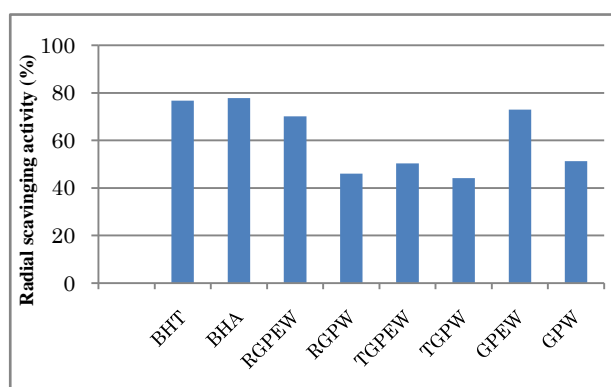


Fig. 2 Free radical scavenging ability (DPPH 2,2 - diphenyl -1-picrylhydrazyl) of extracts of ginger, galangal, BHT and BHA (1mg/ml)

Fig 2 showed the ability of removing DPPH free radicals of extracts of ginger and galangal extracted with ethanol/water solvent were rather high (70.16% and 72.9%) and roughly two times higher than BHT and

BHA antioxidants, while the extracts of small rhizomes variety ginger and big rhizomes variety ginger and galangal extracted with water have the ability to remove DPPH radicals were slightly low (44.08 to 51.25%).

### 3.1.3. Evaluation of the antibacterial ability

Antibacterial ability of the extracts of ginger, galangal was reported a lot and they have ability to resist some microorganisms groups causing contamination and capable of causing disease and toxins in food (JirawanOonmetta-areea, et al., 2006). With a desire to know whether these extracts having the ability to resist or not the microorganism groups causing contamination in seafood, especially squid, we evaluated antibacterial ability of these extracts against microorganisms such as *Staphylococcus aureus*, *E. coli*, *Vibrio parahaemolyticus*, *Salmonella*. The results were shown in the table 1:

Table. 1 Resistance ability against microorganisms of the extracts of ginger, galangal

Type of extract	Diameter of antibacterial ring (D-d) mm			
	With <i>S. aureus</i>	With <i>E. coli</i>	With <i>Salmonella</i>	With <i>V. parahaemolyticus</i>
RGPEW	29	29.5	24	21
TGPEW	22	20	20	18
GPEW	28	24	25	24

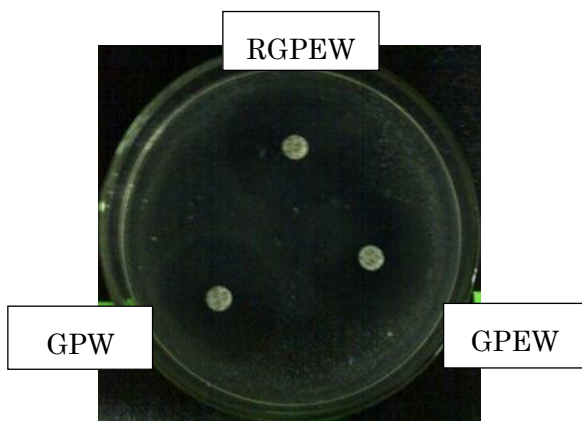


Fig. 3 Diameter of antibacterial ring with *E.coli* of the ginger, galangal extract

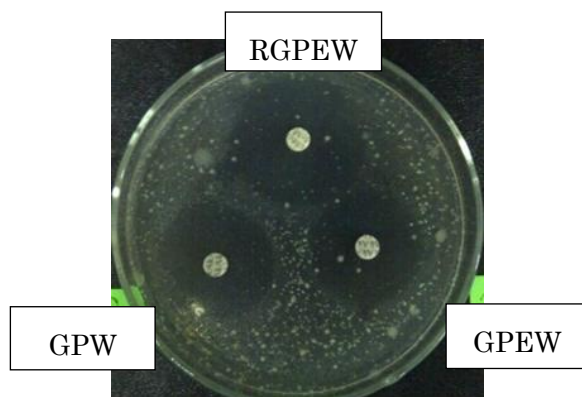


Fig. 4 Diameter of antibacterial ring with *S. aureus* of the ginger, galangal extracts

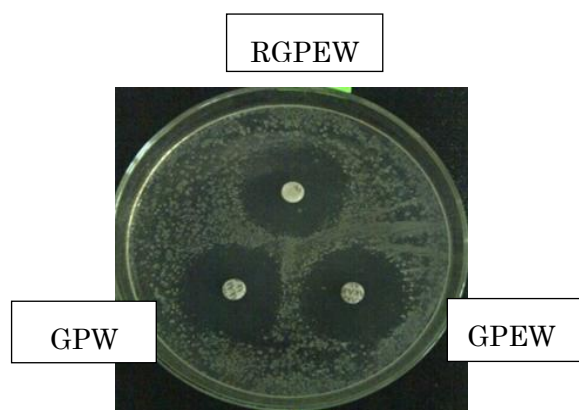


Fig. 5 Diameter of antibacterial ring with *Vibrio parahaemolyticus* of the ginger, galangal extract

The results showed that ginger extracts had better antibacterial ability against *S.aureus* and *E.coli*, and galangal had better antibacterial ability against *Salmonella* and *Vibrio parahaemolyticus*.

### 3.2. Research on preservation of squid with extracts of ginger, galangal

From the research results in 3.1, we chose the appropriate and effective extracts for preserving squids, they are extracts from ginger of small rhizomes variety and galangal because they have the best antioxidant and antibacterial ability.

Conducted a combination of these extracts to process and preserve squid according to the methods in 2.2 as follow:

- Control sample of squid: storage in cold condition without processed with extracts of ginger and galangal
- Squid sample processed with combined of Re ginger extracted with ethanol/water solvent 50% (v/v) and galangal extracted with water: (RGPEW + GPW)
- Squid sample processed with combined of Re ginger extracted with water and galangal extracted with water: (RGPW+GPW)
- Squid sample processed with combined of Re ginger extracted with ethanol/water solvent 50% (v/v) and galangal extracted with ethanol/water solvent 60% (v/v): (RGPEW+GPEW)
- Squid sample processed with combined of Re ginger extracted with water and galangal extracted with ethanol/water solvent 60% (v/v): (RGPW+GPEW)

The results are expressed in physicochemical and microbiological indicators during preservation processes of raw squid up to 13 days are shown in the following of Fig 6 to Fig 9:

Physiochemical changes of squid samples showed that the control sample had speedily increasing pH and  $\text{NH}_3$  content, which presented a quickly spoilage after 4 days of reservation. Meanwhile, these 2 parameters of the 4 squid samples preserved by ginger, galangal extracts changed quite slowly, the sample using 2 extracts from Re ginger and solely by ethanol/water

solvent was the most stable in pH and  $\text{NH}_3$ , followed by sample using Re ginger extracted by ethanol/water solvent combined with galangal extracted by water.

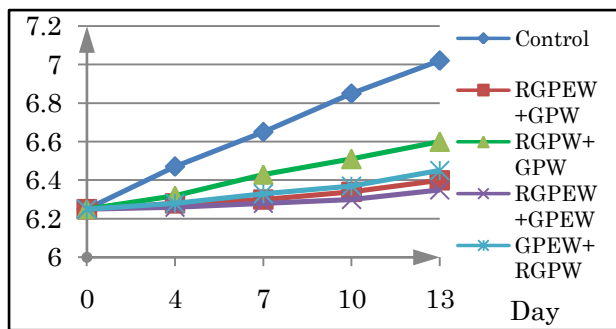


Fig. 6 pH of raw squids processed with extracts of ginger and galangal

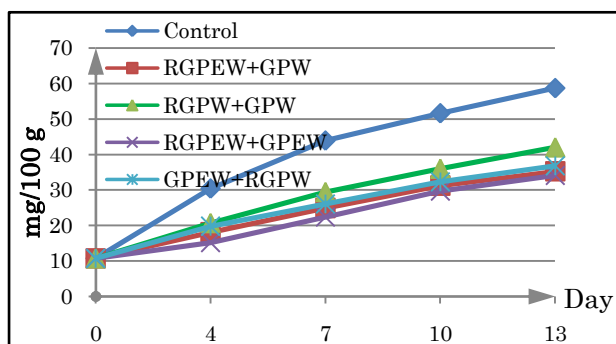


Figure 7.  $\text{NH}_3$  content of raw squids processed with extracts of ginger and galangal

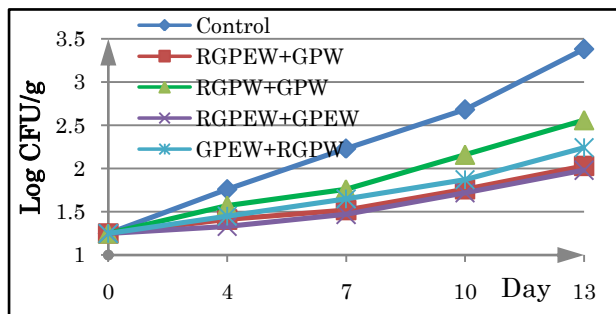


Fig. 8 Number of *Enterobacteriaceae* of squids processed with extracts of ginger and galangal

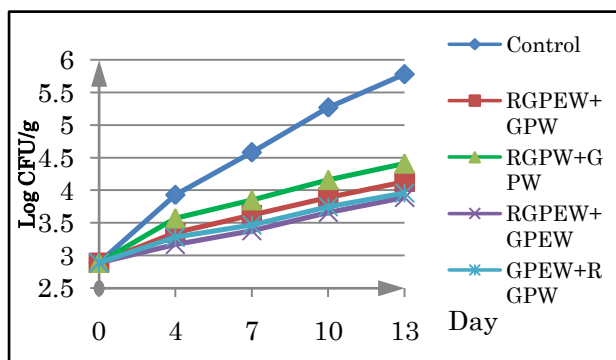


Fig. 9 Number of *S. aureus* of squids processed with extracts of ginger and galangal

The results of Figures 8 and Fig. 9 showed good inhibit ability to bacteria group of *S. aureus* and *Enterobacteriaceae* of the squid samples preserved with extracts of ginger, galangal in compared with the control sample. After 13 days of storage in covered ice to maintain  $0-2^\circ\text{C}$ , the squid samples processed and preserved with extracts of ginger and galangal extracted with ethanol (RGPEW + GPEW) having the slowest increase in number *S. aureus* and *Enterobacteriaceae*, following by the samples preserved with the ginger extracted with ethanol/water combined with galangal extracted with water (RGPEW + GPW) and ginger extracted with water combined with galangal extracted with ethanol/water (GPEW+RGPW) and they 2 samples processed with combined extracts (RGPEW+GPEW) and (RGPEW+GPW) attained the TCVN after 13 days of storage.

The sensory evaluation of color, flavor and texture showed the squid samples processed and preserved with combined extracts kept color, flavor, structure close to the original raw squid sample.

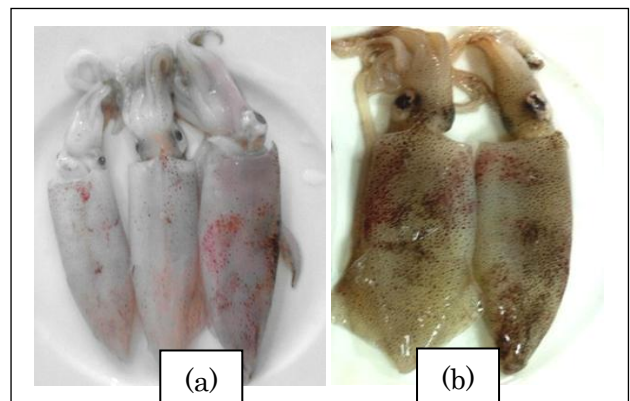


Fig. 10 Picture of squid after 13 days of storage when processed with extracts of ginger and galangal at  $0-2^\circ\text{C}$  with ice (a), and the control sample after 5 days of storage at  $0-2^\circ\text{C}$  with ice (b).

The results of preservation of cuttlefish samples are not significantly different when compared to the results of preservation of squid samples.

## CONCLUSION

The results of evaluating polyphenol components, antioxidant and antimicrobial ability of the extracts from two varieties of ginger and one variety of galangal currently widely planted in Vietnam showed that the Re ginger when extracted with ethanol/water 50% (v/v) had the highest polyphenol content (0.4595 mg/ml equivalent 18.38 mg/g dry powder), rather high antioxidant ability (radical scavenging activity 70.16%) and good antibacterial ability against *S. aureus*, *E. coli*; the galangal extracts with ethanol/water 60% (v/v) having the highest antioxidant ability (radical scavenging activity 72.9%), and best antibacterial ability against



*Salmonella* and *Vibrio parahamolyticus*. The extracts of Trau ginger had much less effect.

Having used combined extracts for preserving effectively fresh squid and cuttlefish after fishing, the results showed the extract of Re ginger and galangal extracted with ethanol/water solvent (RGPEW + GPEW) had the best preservation effectiveness following by the extract of ginger extracted with ethanol/water solvent mixed with galangal extracted with water (RGPEW+GPW), these two extracts could preserve squid and cuttlefish up to 13 days at 0-2°C, the quality was not inferior to that of freshly caught squid and cuttlefish.

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