

*Research Article*

## **Effect of herb and spice pastes on the quality changes in minced salmon flesh waste during chilled storage**

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### **Abstract**

The effects of the antioxidant and antibacterial activities of herb and spice pastes, holy basil and pepper-garlic, on minced salmon flesh waste stored at 4<sup>0</sup>C was determined over 12 days. Physical, chemical, microbiological and sensory analyses were performed to investigate quality changes and to determine the shelf-life of the products. The initial pH of the control sample was significantly lower (p<0.05) than the other treatments added with the pastes throughout the storage. However, the pH of all samples slightly decreased as storage time increased. The total volatile nitrogen values (TVB) of all samples were not over the standard limit (<35 mg/100 g sample) during storage. The trimethylamine (TMA) of the control was 1.11±0.33 at day 6 while samples added with the paste, particularly pepper-garlic paste, had very low values (<0.7 mg/100 g sample) throughout the storage. Rancidity, measured as thiobarbituric acid reactive substances (TBARS), of the sample with holy basil paste added was higher than the control. The sample with pepper-garlic paste added was lower compared with the control during storage. However, both pastes showed significant differences in inhibiting microbial growth, particularly mesophilic bacteria, leading to an extension of the shelf-life, compared with the control, of at least 3 days. Pepper-garlic paste offers an attractive means to preserve the shelf-life of minced salmon flesh waste during chilled storage.

**Keywords:** food waste, natural antioxidant, antimicrobial, TMA, TBARS, quality assessment, shelf-life, Thailand.

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## Introduction

Salmon is recommended as having the highest content of polyunsaturated fatty acids, (PUFA), especially eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), compared with other food such as fish and other seafood [1]. When whole salmon is filleted, more than half is wasted, and is sold as cheap feed for pets or ends up as waste. Recently, most companies have normally had to pay for disposal of such by-products. The challenge is to achieve profitable processing of this waste. Therefore, there is increasing interest in processing by-products, such as using meat from fillet trimming as sandwich spreads, sauces and soups.

In addition, the by-products of salmon are also reported as a high quality and good source of omega-3 fatty acid. They can be converted into a number of products [2, 3]. However, the shelf life of refrigerated seafood generally ranges from 2 to 14 days [4]. The shelf life after refrigeration is limited due to the rapid growth of microorganisms as well as sensory spoilage [5, 6, 7]. The addition of chemical additives (such as preservatives, antioxidants, colourants) to extend shelf life has frequently been associated with certain health problems. These include allergies and other more serious illnesses, such as the initiation of carcinogenesis [8]. Scientists have become interested in using antioxidants from natural sources and several researchers have shown that various tissues from plants contain high concentrations of natural phenolic phytochemicals, including flavonoids [9, 10, 11].

Polyphenolics are one of the compounds that are found in both edible and inedible plants and herbs/spices and it could be the source of a good antioxidant agent. The antioxidant activity of phenolic compounds in herbs and spices is mainly due to their redox properties and chemical structures. These can act as reducing agents, free radical scavengers and Fe<sup>2+</sup> chelators or quenchers in the formation of singlet oxygen [12, 13, 14]. Thus phenolics are of increasing interest in the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food [15]. Moreover, many studies have reported the antimicrobial activities of spices and herbs or essential oils [16]. The antimicrobial activity of plant extracts may be located in a variety of different components, including aldehyde and phenolic compounds [17, 18] as well as sulphur [19, 20].

The objective of this study was to determine the antimicrobial and antioxidant qualities of herbs/spices used in holy basil and pepper-garlic pastes in minced salmon. A further objective was to evaluate the effectiveness of using these pastes to prolong the shelf life of minced salmon products.

## Materials and Methods

### *Plant materials*

The holy basil paste consisted of garlic (*Allium sativum* L.), holy basil (*Ocimum sanctum* Linn) and bird chili (*Capsicum frutescens* Linn). The pepper-garlic paste consisted of a blend of garlic and black pepper (*Piper nigrum* Linn). All fresh ingredients were purchased from local markets while pepper seed was bought in packed form from a supermarket in Haad Yai, Thailand.

### ***Minced salmon***

Trimmed frozen salmon pieces were obtained from a salmon processing plant located in Songkhla province. They were taken to the laboratory within 1 hour before being subjected to thawing, skinning and mincing for further study.

### ***Paste preparation***

The holy basil paste used was a combination of holy basil (18%), garlic (46%) and bird chili (36%). The pepper-garlic paste consisted of garlic (75%) and black pepper (25%). They were blended in a Philips HR-2068 blender (Thailand).

### ***Application of the paste to minced salmon***

The individual pastes were prepared as mentioned above and were added to minced salmon in the ratio of 1:4 and 1:5 (mince: paste). They were then chopped with a silent cutter for 2 minutes and packed in a nylon/LLDPE bag at a thickness of 5.0 mm. The control sample was minced salmon without the paste. The samples were stored in a refrigerator at 4°C for 12 days and every three days the samples were analyzed to determine the chemical, physical, microbiological and sensory changes.

### ***pH***

Ten-g of each sample was homogenized with 10 ml distilled water [21] before being measured with a pH meter (Thermo Orion, Model 230,)

### ***Colour***

The colour values, L\*, a\* and b\*, were measured using a colorimeter (Hunter Lab, Model ColorFlex) with 10° standard observers, illuminant D65, a 5 mm aperture for illumination and 8 mm for measurement. Five replicated measurements were taken for each sample, following the guidelines for colour measurements set by the American Meat Science Association [22].

### ***Lipid oxidation***

Lipid oxidation was assessed in triplicate using the 2-thiobarbituric acid reactive substances (TBARS) method of Lopez *et al.* [23]. Briefly, 10 g sample was blended with 50 ml distilled water for 2 minutes then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 ml of distilled water. This was added to a distillation tube containing 2.5 ml 4 N HCl and a few drops of antifoam agent. Five-ml of 0.02 M 2-thiobarbituric acid in 90% acetic acid glacial (a TBA reagent) was added to a vial containing 5 ml of distillate and mixed well. The vials were capped and heated in boiling water for 30 minutes and cooled to room temperature. The absorbance was measured at 532 nm using a spectrophotometer (Shimadzu, UV-16001, Australia).

### ***TMA and TVB values***

TMA and TVB values were measured following the methods of Hasegawa [24]. The sample (2g) was homogenized with 4% trichloroacetic acid at a ratio of 1:4 (w/v). The homogenate was filtered through Whatman No 41 paper before being subjected to analysis in accordance with the Conway method.

### ***Microbiological analysis***

The sample (25 g) was mixed with 225 ml of sterile 0.01% peptone water in a Stomacher 400 (Colworth, London, UK) for 1 minute. Decimal dilutions were made using sterile 0.01% peptone water to obtain the proper dilution. Mesophilic bacteria counts were determined by plate count agar (Merck Laboratories, Darmstadt, Germany) with incubation at 37°C for 24–

48 hours. The psychrophilic bacteria counts were also determined by plate count agar (Merck Laboratories, Darmstadt, Germany) with incubation at 4°C for 7 to 10 days as described by BAM [25].

## Results and Discussion

### Change in pH value

The effect of pastes and storage time on the pH of the minced salmon during storage at 4°C is shown in Figure 1. The initial pH value of the control (6.18) was significantly ( $P < 0.05$ ) lower than that of samples with the pastes added throughout the storage. Generally, the changes in the products pH value may be due to the acid which is a common metabolite from a number of bacteria. These may include lactic acid bacteria, Enterobacteriaceae and *P. phosphoreum* [26], and the basic compounds such as  $\text{NH}_3$ , TMA and DMA which are developed from a combination of microbial and autolytic actions. This implied that there were higher lactic acid bacteria in the control sample compared with the minced salmon with the paste containing the garlic as an antibacterial agent [27, 28].

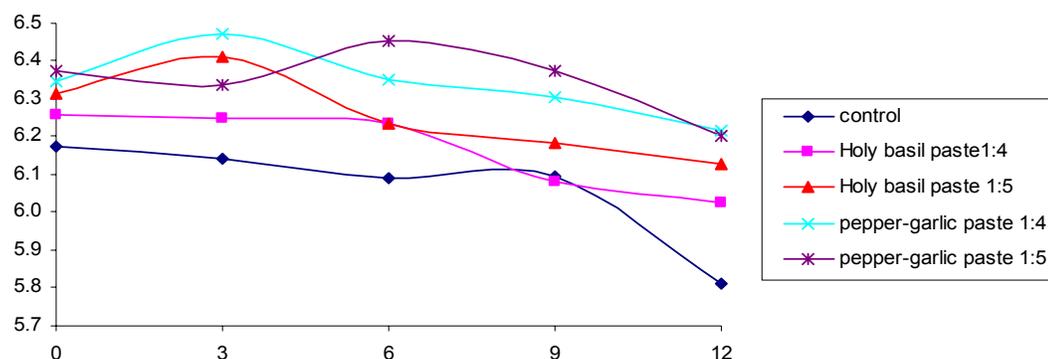
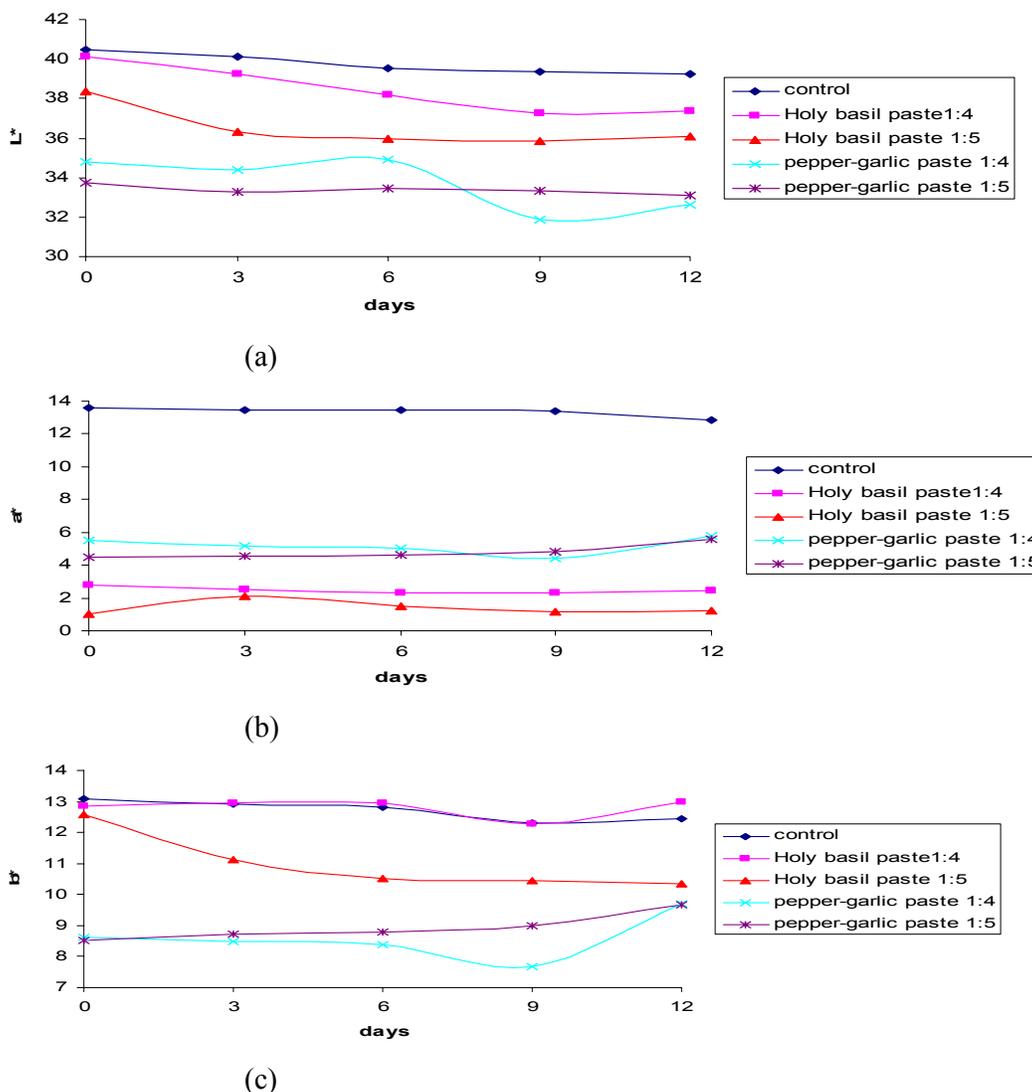


Figure 1. Effect of the pastes on pH values of salmon during storage at 4°C.

### Colour

Three colour parameters, lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ), were used to investigate the changes in colour of the salmon. In all samples lightness,  $L^*$ , decreased with storage time and the highest values of  $L^*$  and  $a^*$  were in the control sample (Fig. 2) compared with samples with the paste added. This was due to the effect of the paste, particularly the pepper-garlic treatment. In addition, it was found that when the proportion of paste was higher, the values the  $L^*$  and  $a^*$  were lower. For yellowness, the pepper-garlic paste had a lower  $b^*$  value than the holy basil paste and the control, perhaps because of black colour from the black pepper powder.

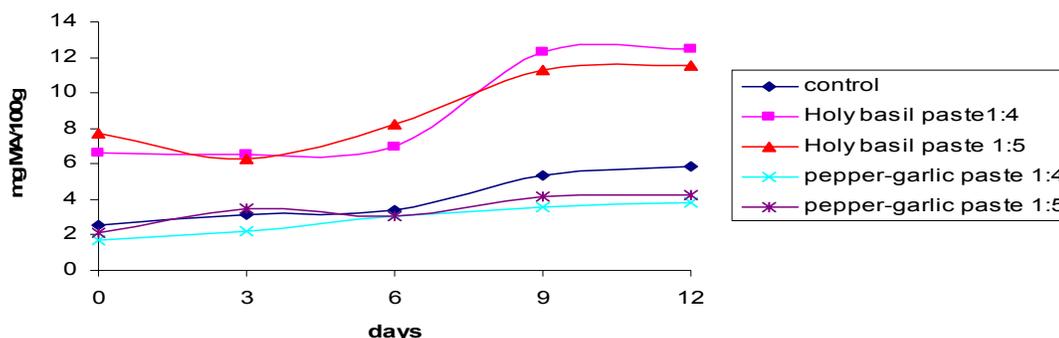


**Figure 2. Effect of the pastes on colour parameters of salmon during storage at 4°C.**  
(a): L\*value, (b): a\* value (c): b\*value

### Lipid oxidation

The TBARS values indicate the content of secondary lipid oxidation products, mainly aldehydes (or carbonyls), which contribute to off-flavours in oxidized meat and meat products [29, 30]. The effect of the pastes on the lipid oxidation (TBARS values) of minced salmon refrigerated for 12 days is shown in Figure 3. The TBARS values increased in all samples over time, particularly in the control sample and the sample with holy-basil paste added at a ratio of 1:4. From previous experiments, it was found that holy basil possessed good DPPH radical scavenging activity in a vitro system [31]. The TBARS values of the holy basil paste samples at ratios of 1:4 and 1:5 were significantly higher than the control ( $P \leq 0.05$ ). This may be due to the pro-oxidant, chlorophyll, which is in agreement with the findings of Juntachote, *et al.* [30]. This was also similar to the findings of Endo *et al.* [31], who reported that chlorophylls and their derivatives promote oxidation of lipids during storage. He and Shahidi [32], have shown that the antioxidant effect of green tea extracts in white muscles of mackerel might be markedly influenced by the presence of chlorophyll and other impurities.

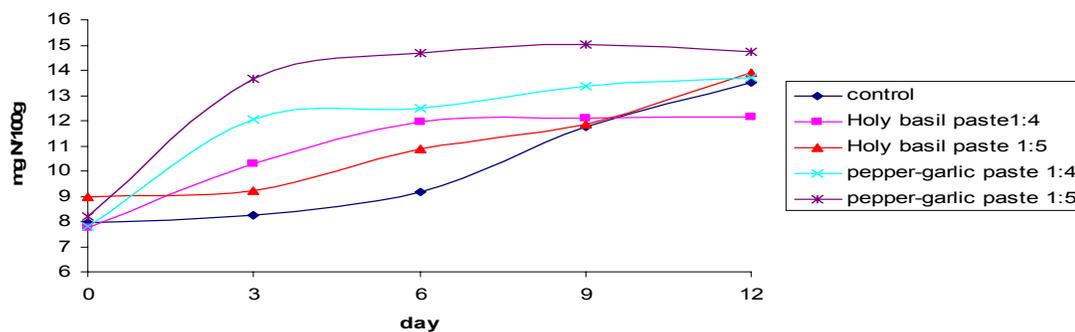
However, the TBARS values of samples added with pepper-garlic pastes at ratios of 1:4 and 1:5 were significantly lower than the control. This was due to the antioxidant compounds, alliin, diallyl sulphide, allyl sulphide and propyl sulphide derived from garlic [33] and piperine in black pepper [34]. This result suggests that pepper-garlic paste could retard lipid oxidation immediately during mixing and during storage. Moreover, the oxidative activity of pepper-garlic pastes was related to their concentration as higher concentration lowers the TBARS content (Fig 3). From the results it could be deduced that garlic-pepper paste was suitable for retarding lipid oxidation in food products throughout storage. This result was in agreement in Tipsrisukond, *et al.* [35]. They observed that ground black pepper added at 0.4% w/w to ground pork had a significant antioxidative effect. Martinez, *et al.* [36] found that black pepper at concentrations of at least 0.5% could retard TBARS values to remain below 1 mg malonaldehyde/kg sample after 16 days of storage in dark conditions.



**Figure 3. Effect of the paste and concentration on 2-thiobarbitulic acid reactive substances (TBARS) values in mince stored at 4°C.**

#### **Total volatile base nitrogen and trimethylamine values**

TVB-N and TMA are very useful indices for spoilage in fresh and lightly preserved seafood [38]. In this study, the initial TVB-N value (mg N/100 g) in minced salmon was in the range 7.973±1.45 to 9.036±0.566 mg N/100 g. These increased with the storage time but were still under the standard limit (less than 35 mg N/100 g) as shown in Figure 4. Surprisingly, the control sample had the lowest TVB-N value during storage; this may due to some protease enzymes remaining in the ingredients used in the pastes.



**Figure 4. Effect of the paste on total volatile base nitrogen (TVB-N) values in minced salmon stored at 4°C.**

The changes in the TMA content in minced salmon stored under refrigeration at 4°C for 12 days are shown in Table 1. TMA is directly related to the microbial spoilage in various species of fish during their storage under refrigerated conditions [37]. Initial TMA values

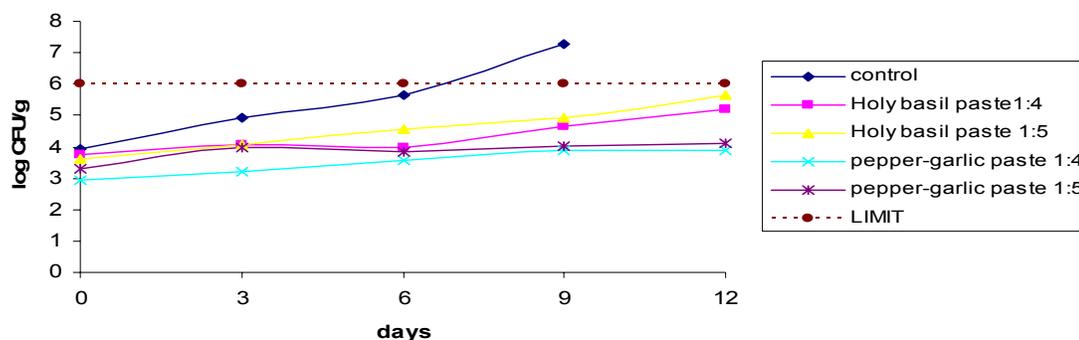
could be detected in only the control sample, while samples with the holy-basil paste added at a ratio of 1:5 had a minor TMA value,  $0.669 \pm 0.001$ , at the end of storage. There was no TMA detected in any sample treated with garlic-pepper pastes and in the holy-basil paste at the higher concentration. This indicated that that bacteria providing TMAOase were inhibited by pepper and/or garlic. Siripongvutikorn, *et al.* [27] reported that garlic could inhibit both Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative (*Escherichia coli* O157:H7 and *Pseudomonas fluorescence*) bacteria.

**Table 1. Effect of the paste on trimethylamine (TMA) values in minced salmon stored at 4°C.**

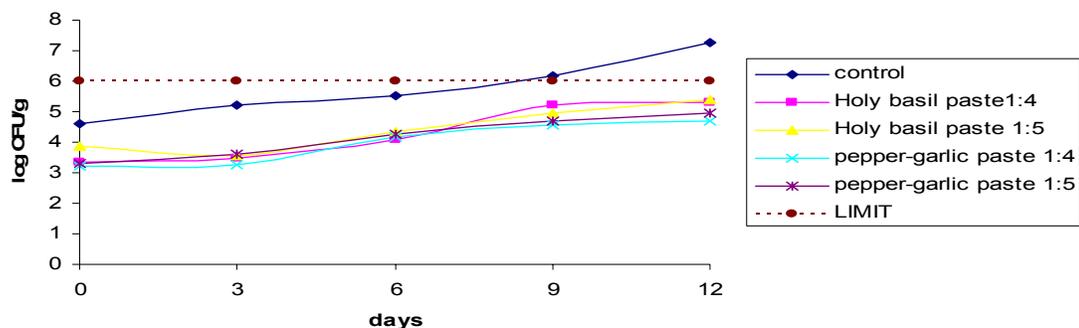
	Control	Holy basil paste 1:4	Holy basil paste 1:5	Pepper-garlic paste 1:4	Pepper-garlic paste 1:5
0	-	-	-	-	-
3	-	-	-	-	-
6	$1.109 \pm 0.334$	-	-	-	-
9	$1.879 \pm 0.354$	-	-	-	-
12	$2.107 \pm 0.627$	-	$0.669 \pm 0.001$	-	-

### Microbiological changes

Mesophilic bacteria of minced salmon with and without the addition of the pastes and refrigerated for 12 days are shown in Figure 5a. In general, the highest microbial growth was obtained from the control samples, while the lowest microbial development was observed in samples treated with pepper-garlic paste at a ratio of 1:4. When aerobic plate counts (mesophilic bacteria) reached  $6 \log_{10}$  CFU/g, the food product was assumed to be at or near spoilage [38, 39]. In this study, microorganisms exceeding 6 log CFU/g were observed in the control sample after 9 days storage while other samples were under the standard level, even after 12 days.



(a)



(b)

**Figure 5. Effect of the paste on microorganism values in mince stored at 4°C.**

(a): mesophilic bacteria; (b) psychrophilic bacteria

Changes in the psychrophilic bacteria of minced salmon are shown in Figure 5b. The initial count in the control was 4.63 log<sub>10</sub> CFU/g which was higher than in the other samples. In addition, the control sample was highest and the mesophilic bacterial count was over 6 log CFU/g after 9 days of storage. The sample with holy basil paste added tended to have a higher psychrophilic population than the sample treated with pepper-garlic paste. This may be due to the effects of the garlic and pepper content. As described in the paste preparation section the holy basil paste was low in garlic content and had no black pepper; both of these ingredients were reported as natural preservative agents [40]. Sulphur compounds are the main active antimicrobial agents [41]. Therefore, the sample with pepper-garlic paste added, particularly at a ratio of 1:4, was most suitable for preserving minced salmon during refrigeration.

## Conclusions

According to the results of this study, pepper-garlic paste exhibited a protective effect against lipid oxidation, but holy basil paste increased lipid oxidation in minced salmon during storage at 4°C for 12 days. Adding both pastes at ratios of 1:4 and 1:5 was sufficiently effective in inhibiting microbial growth and retarding TMA values. Therefore, further study of using pepper-garlic paste as a natural additive in order to increase the shelf life of food by preventing microbial growth and lipid oxidation is recommended. In so doing sensory evaluation should also be taken into account to confirm the acceptability of the product.

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