

*Research Article*

## **Darkening prevention of fermented shrimp paste by pre-soaking whole shrimp with pyrophosphate**

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### **Abstract**

The effect of pre-soaking whole shrimp (*Acetes vulgaris*) with pyrophosphate (PP) on darkening prevention of Thai fermented shrimp paste was investigated. Whole shrimp were soaked with 2% PP (w/v) at a ratio of solution:shrimp of 1:2 (v/w) at 4°C for 2 h and subsequently drained for 10 min at 4°C. Control was run in the same manner but distilled water was used instead of PP. Pre-treated shrimp were subjected to shrimp paste fermentation for 30 days. TCA-soluble peptide of both shrimp pastes increased throughout the fermentation time ( $P < 0.05$ ). During the same fermentation period, soaking with PP had no effect on TCA-soluble peptide of shrimp paste when compared with control ( $P > 0.05$ ). The thiobarbituric acid reactive substances (TBARS) value of control shrimp paste was generally higher than treated shrimp paste throughout the fermentation time. Higher astaxanthin content and  $a^*$  value corresponded with the lower  $b^*$  value,  $A_{294}$  and  $A_{420}$  of shrimp paste processed from treated shrimp ( $P < 0.05$ ), revealing less darkening when compared to control. Sensory evaluation suggested that shrimp paste produced from shrimp pre-soaked with PP showed a superior colour and overall acceptance score to control shrimp paste ( $P < 0.05$ ). In addition, no difference in odour score was found between control and treated shrimp pastes ( $P > 0.05$ ). Therefore, pre-soaking whole shrimp with PP can be used to retard the dark discolouration of shrimp paste without negative effects on protein hydrolysis and off-odour development.

**Keywords:** additives, TBARS, *Acetes vulgaris*, sensory evaluation, condiments, Thailand.

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### **Introduction**

Thai traditional fermented shrimp paste named *Kapi* is widely consumed in Southern Thailand as a seasoning ingredient. Similar forms of this paste are also commonly used in Southeast Asian cuisine where it is known as *Bagoong alamang* in the Philippines, *Mam ruoc* in Vietnam, *Terasi* in Indonesia, *Ngapi* in Myanmar and *Belachan* in Malaysia. This product is typically prepared

from the sergestid shrimp (*Acetes vulgaris* or *Mesopodopsis orientalis*), mixed with salt at an appropriate ratio, sun-dried to decrease the moisture content and then blended thoroughly (Faithong *et al.*, 2010). The paste is compacted and allowed to ferment in an earthen jar until the desired aroma has developed (Phithakpol, 1993). Protein hydrolysis during fermentation of shrimp paste is mediated by microbial or indigenous proteases. This reaction yields short chain peptides and free amino acids, resulting in the formation of typical flavour and taste (Faithong *et al.*, 2010). During fermentation, many reactions might involve in the development of colour of fermented shrimp paste. The degree of browning reaction might affect the acceptability of the paste, where pink-brown colour development is considered desirable. Reddish pink to orange colour of shrimp paste might result from the release of the free natural pigment, astaxanthin, from protein-bound form as a result of proteolysis. Browning development can occur from both enzymatic and non-enzymatic reactions. Active polyphenoloxidase and Maillard reaction might cause the formation of brown pigment. In addition, the oxidation of free astaxanthin can result in the pale discolouration of products. Aldehydic lipid oxidation products seemed to enhance the Maillard reaction. Therefore, the degree of browning or darkening of fermented shrimp paste might result from a combined effect of these colour reactions together with the decrease in astaxanthin content. As a consequence, the addition of some safe food additives to retard these phenomena could be a promising means to enhance the quality and acceptability of fermented shrimp paste. Phosphate has been used to retard the oxidation of lipid in muscle food (Molins, 1990) as well as to reduce the black spot formation in refrigerated shrimp (Thepnuan *et al.*, 2008). Hence, the objective of this study was to investigate the effect of pre-soaking whole shrimp (*Acetes vulgaris*) with PP on darkening prevention of Thai fermented shrimp paste.

## Materials and Methods

### Materials

Small shrimp (*Acetes vulgaris*) were purchased from a local market in Thasala, Nakhon Si Thammarat, Thailand. The shrimps were transported to the School of Agricultural Technology, Walailak University in ice with a shrimp/ice ratio of 1:2 (w/w) within 20 min. Upon arrival, the shrimp were separated into 2 groups and subjected to soaking with distilled water (control) or 2% PP (w/v) at a ratio of solution:shrimp of 1:2 (v/w) at 4°C for 2 h and drained for 10 min at 4°C. To conventionally prepare fermented shrimp paste, soaked shrimp (10 kg) were mixed with solar salt (1.5 kg), kept in a plastic bag for 12 h at ambient temperature (27-35°C), sun-dried for 8 h and coarsely blended. The blend was kept in a plastic bag for 36 h, sun-dried for 8 h and blended thoroughly to obtain the homogenous paste. The paste was transferred to earthen jars and subjected to fermentation for 30 days at ambient temperature. During fermentation, the earthen jars were covered with a plastic bag and aluminum lid to prevent the contamination of foreign materials as well as insects. The shrimp paste samples were randomly taken from 3 different lots at day 0, 5, 10, 15, 20, 25 and 30 of fermentation for chemical and physical analysis.

### Methods

#### Determination of TCA-soluble peptides

TCA-soluble peptides were determined according to the method described by Morrissey *et al.* (1993). Each sample (3 g) was homogenized with 27 ml of 5% TCA (w/v) for 1 min at room temperature using an IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was kept in ice for 1 h and centrifuged at 7,500×g for 5 min. TCA-soluble peptides in the supernatant were measured according to the Lowry method (Lowry *et al.*, 1951) and expressed as μmole tyrosine/g sample (Morrissey *et al.*, 1993).

#### *Determination of thiobarbituric acid-reactive substances (TBARS)*

TBARS assay was performed as described by [Buege and Aust \(1978\)](#). Each sample (0.5 g) was homogenized with 2.5 ml of a solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N HCl using an IKA Labortechnik homogenizer (Selangor, Malaysia). The mixture was heated in a boiling water bath (95-100°C) for 10 min to develop a pink colour, cooled with running tap water and centrifuged at 3,600×g at 25°C for 20 min using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). The absorbance of the supernatant was measured at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane at concentrations ranging from 0 to 10 ppm. TBARS was calculated and expressed as mg malonaldehyde/kg sample.

#### *Determination of astaxanthin content*

Astaxanthin content was determined according to the method of [Holanda and Netto \(2006\)](#) with slight modification. Each sample (1 g) was homogenized with 10 ml of a petroleum ether-acetone-water mixture (15:75:10 v/v/v) for 2 min at room temperature using an IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was kept at room temperature for 10 min and then centrifuged at 3,500×g for 30 min at 25°C using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). Solvent-extracted astaxanthin was quantified by measuring absorbance at 470 nm and the coefficient of extinction used was  $E_{1\%}^{1\text{cm}} = 2,400$ . Astaxanthin content was expressed as mg/100 g sample.

#### *Determination of UV-absorbance and non-enzymatic browning*

The UV-absorbance and non-enzymatic browning of samples were measured according to the method of [Ajandouz et al. \(2001\)](#). Each sample (5 g) was homogenized with 10 ml of distilled water using an IKA Labortechnik homogenizer (Selangor, Malaysia). The homogenate was centrifuged at 3,500×g for 20 min at 4°C using a RC-5B plus centrifuge (Sorvall, Norwalk, CT, USA). Appropriate dilution of the supernatant was made using distilled water and the absorbance was measured at 294 and 420 nm using a spectrophotometer (UV-1601, Shimadzu, Japan).

#### *Determination of colour*

The colour of shrimp paste was determined by measuring a\* (+red to –green component) and b\* (+yellow to –blue component) values using a colorimeter (Juki Corp, Tokyo, Japan). Samples were placed into a 5-cm diameter glass petri dish and illuminated with D65-artificial daylight (10° standard angle).

#### *Sensory evaluation*

Shrimp pastes fermented for 30 days were evaluated for acceptance by an untrained 30 member panel. The panelists were under-graduate students in the Food Technology Program of age ranging from 19 to 21 years, School of Agricultural Technology, Walailak University. A nine-point hedonic scale, in which a score of 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely, was used for evaluation ([Chamber and Wolf, 1996](#)). The panelists evaluated each sample for colour, odour and overall liking.

#### *Statistical analysis*

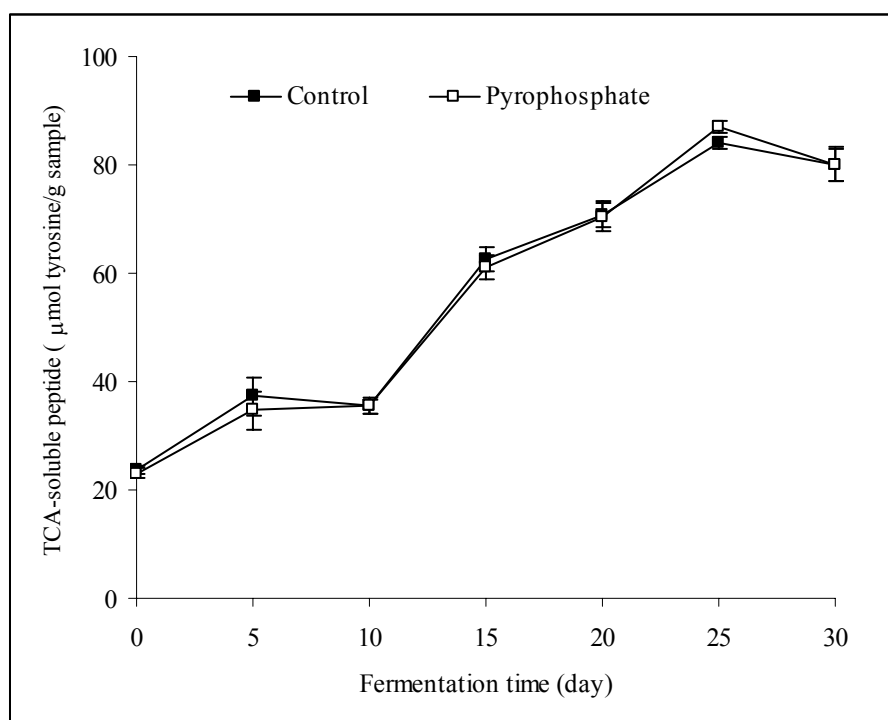
Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test ([Steel and Torrie, 1980](#)). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 8.0 for windows, SPSS Inc., Chicago, IL).

## Results and Discussion

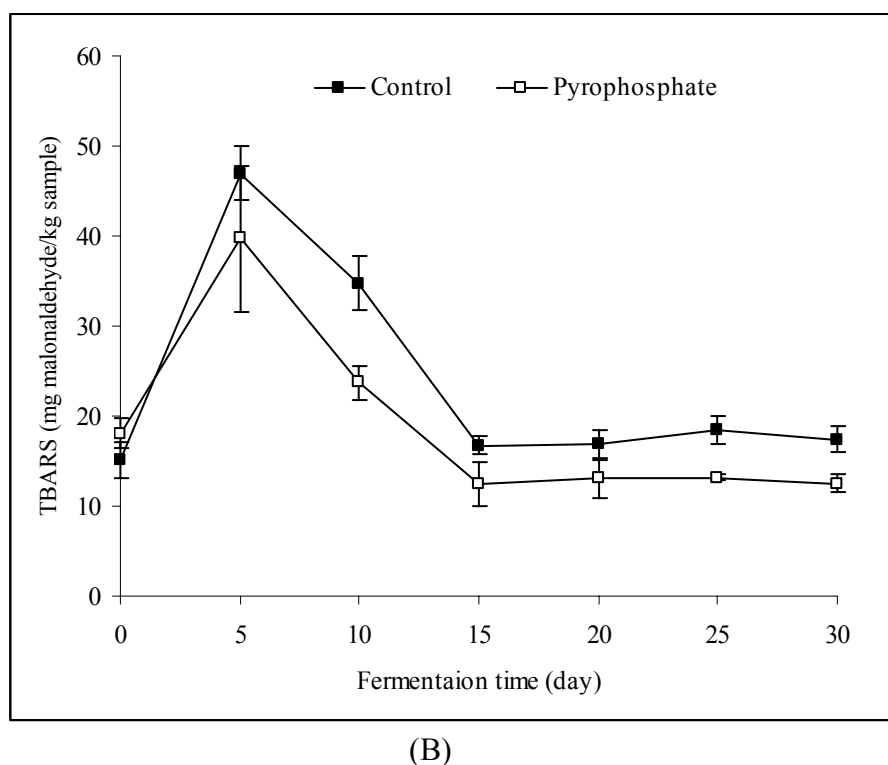
### *Changes in TCA-soluble peptide content and TBARS value of shrimp paste during fermentation*

Changes in TCA-soluble peptide content and TBARS value of fermented shrimp paste produced from shrimp treated with distilled water and 2% PP during 30 days fermentation are depicted in Figures 1(A) and 1(B), respectively. TCA-soluble peptide of both shrimp pastes increased throughout the fermentation time ( $P < 0.05$ ; Fig. 1(A)). This result suggested that protein hydrolysis in both treated shrimp and control occurred continuously during fermentation. At the same fermentation period, soaking with PP had no effect on TCA-soluble peptide of shrimp paste when compared with control ( $P > 0.05$ ). This result indicated that soaking with PP had no effect on protein hydrolysis during shrimp paste fermentation.

The TBARS value of control shrimp paste was generally higher than treated shrimp paste throughout the fermentation time indicating a higher degree of lipid oxidation in control shrimp paste (Fig. 1B). The result suggested that pre-soaking whole shrimp with PP could effectively retard the oxidation of lipids during fermentation by means of chelating mechanism (Molins, 1990). Lowered TBARS value of PP-treated shrimp paste indicated the lowered formation of aldehydes which was one of the causes of Maillard browning reaction. Therefore, the lower the TBARS observed, the lower the darkening of fermented shrimp paste might be obtained.



(A)

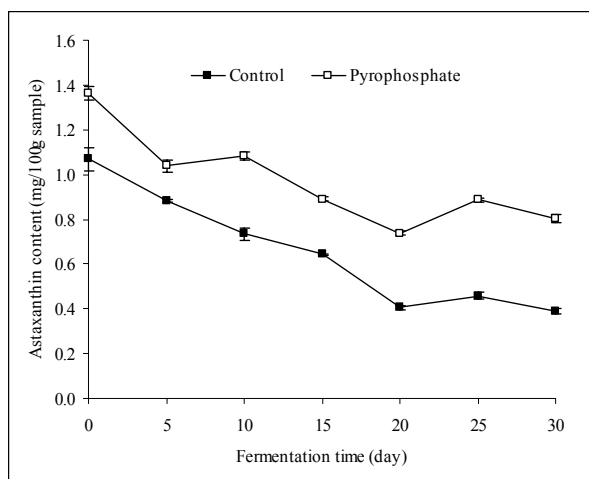


**Figure 1. TCA-soluble peptide content (A) and TBARS value (B) of fermented shrimp paste produced from shrimp treated with distilled water and 2% PP during 30 days fermentation.**

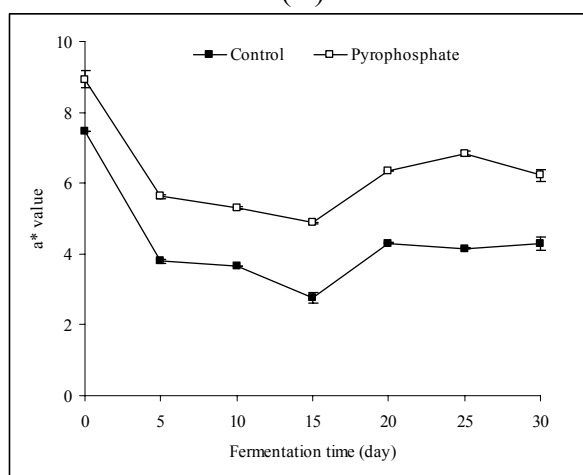
Bars represent the standard deviation from triplicate determinations.

#### ***Changes in astaxanthin content and colour of shrimp paste during fermentation***

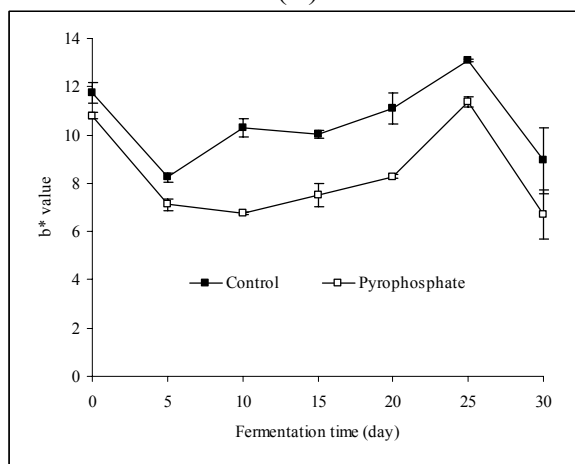
Higher astaxanthin content (Fig.2A) and  $a^*$  value (Fig.2B) corresponded with the lower  $b^*$  value (Fig. 2C),  $A_{294}$  (Fig. 3A) and  $A_{420}$  (Fig.3B) of shrimp paste processed from treated shrimp ( $P < 0.05$ ) revealed the lowered darkening when compared to control. The reduction of astaxanthin content in shrimp paste might be associated with the pale discolouration of the paste caused by the oxidation and/or degradation of astaxanthin during fermentation. The result showed that the astaxanthin content was found to be higher in shrimp paste processed from PP pretreated shrimp than control ( $P < 0.05$ ; Fig. 2A)). It can be postulated that PP can stabilize the astaxanthin in shrimp during shrimp paste fermentation. The higher the astaxanthin content of PP treated shrimp paste, the higher the  $a^*$  (Fig. 2B) value and the lower the  $b^*$  value (Fig. 2C) throughout the fermentation period. The  $A_{294}$  and  $A_{420}$  were used to indicate the formation of intermediate compounds and browning intensity derived from Maillard reaction, respectively (Ajandouz *et al.*, 2001). The lower  $A_{294}$  and  $A_{420}$  of PP treated shrimp paste indicated the lower darkening of that paste (Fig. 3A and 3B). From the results, the formation of dark-coloured compounds in shrimp paste was more retarded in paste produced from PP treated shrimp. Therefore, the use of PP as a soaking agent was possible to prevent the dark discolouration of fermented shrimp paste.



(A)



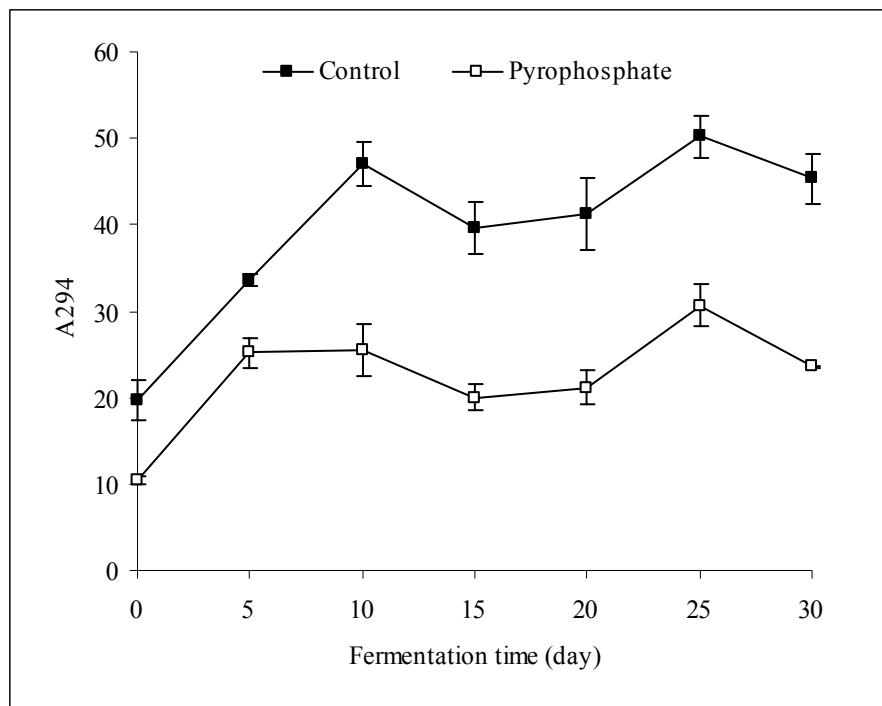
(B)



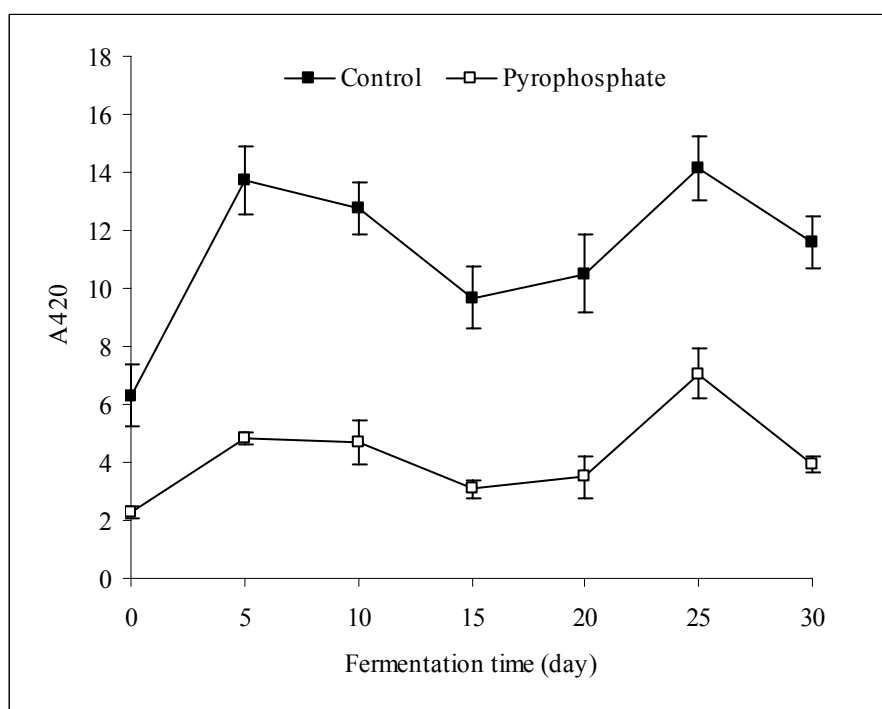
(C)

**Figure 2. Astaxanthin content (A), a\* value (B) and b\* value (C) of fermented shrimp paste produced from shrimp treated with distilled water and 2% PP during 30 days fermentation.**

Bars represent the standard deviation from triplicate determinations.



(A)



(B)

**Figure 3. Absorbance at 294 nm (A) and browning intensity (A<sub>420</sub> nm) (B) of fermented shrimp paste produced from shrimp treated with distilled water and 2% PP during 30 days fermentation.**

Bars represent the standard deviation from triplicate determinations.

**Acceptability of fermented shrimp paste**

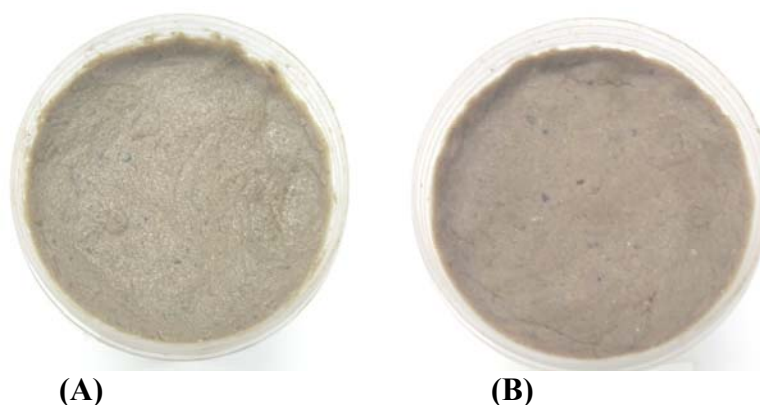
Acceptance scores of shrimp paste produced from shrimp treated with distilled water and 2% PP after 30 days of fermentation are shown in Table 1. It was noted that shrimp paste produced from shrimp presoaked with PP showed a superior colour and overall acceptance score to control shrimp paste ( $P < 0.05$ ; Table 1 and Fig. 4). In addition, no difference in odour score was found between control and treated shrimp pastes ( $P > 0.05$ ). The result suggested that pre-soaking whole shrimp with PP prior to fermentation had no effect on off-odour formation, but it can improve the colour and overall liking of the resulting shrimp paste significantly.

**Table 1. Acceptance score of 30-d fermented shrimp paste produced from shrimp treated with distilled water and 2% pyrophosphate.**

Sample	Score		
	Colour	Odour	Overall liking
Control	3.37±1.92*a**	4.17±2.10a	4.83±2.01a
Pyrophosphate	5.17±2.24b	5.47±1.93a	6.23±1.50b

\*Values are given as mean±SD from 30 determinations.

\*\*Different letters within the same column indicate significant differences ( $P < 0.05$ ).



**Figure 4. Appearance of fermented shrimp paste produced from shrimp treated with distilled water (A) and 2% PP (B) after 30 days fermentation.**

**Conclusion**

Pre-soaking whole shrimp with PP can be used to retard the dark discolouration of shrimp paste without negative effects on protein hydrolysis and off-odour development.

**Acknowledgments**

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