# Phenolic Content and DPPH Radical Scavenging Activity of Yam-containing Surimi Gels Influenced by Salt and Heating[†]

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## ABSTRACT

The factors contributing to the loss of phenolic content and DPPH radical scavenging activity of Taiwanese yam, *Dioscorea alata* Tainung No. 1 (TNG1), in the 20% TNG1-containing pollock surimi gel were investigated. Heating at 90°C for 30 min decreased both the total phenolic content and DPPH radical scavenging activity in 50% ethanolic extract from TNG1, but no significant effect was found for adding 2% NaCl. Blending 20% TNG1 with pollock surimi without heating or adding 2% NaCl could reduce the DPPH radical scavenging activity. For 20% TNG1-containing surimi samples, heating at 90°C for 30 min decreased the total phenolic content, while the combination of the heat treatment and 2% NaCl resulted in further decrease. It was suggested that heat treatment might also cause an interaction between the denatured fish proteins and phenolic compounds in TNG1, thus decreasing the extractability of the phenolic compounds.

***Key words***: yam, surimi, antioxidant, gel-forming, phenolic compounds.

## 1. INTRODUCTION

Yam is one of the staples of many tropical countries. For example, yam is widely grown in west Africa (Waitt, 1963; Coursey & Haynes, 1970). Some yams are also used as medicines in oriental countries to prevent diarrhea and diabetes (Hsu, Chen, Hsu, Chen & Chang, 1984; Yen, 1992). Yam is composed mainly of starch (75-84% of the dry weight) with small amounts of proteins, lipids and most vitamins and is very rich in minerals (Omonigho, 1988; Lasztity, Hidvegi & Bata, 1998). Researches have shown that yam extracts can reduce blood sugar (Undie & Akubue, 1986; Hikino et al., 1986) and blood lipid (Araghiniknam, Chung, Nelson-White, Eskelson & Watson, 1996), inhibit microbial activity (Kelmanson, Jager & Van Staden, 2000; Hu, Dong, Yao, Kobayashi, & Iwasaki, 1996; Hu et al., 1999) and show antioxidative activity (Farombi, Britton & Emerole, 2000; Chan, Hsu, Wang & Su, 2004).

Surimi is a washed fish mince to which cryoprotectants, such as sorbitol and sucrose, are added to maintain protein functionality during frozen storage (Lee 1984; Matsumoto & Noguchi, 1992). Surimi can be further processed into various surimi-based gelled products, such as artificial crab legs and meats, with unique textural properties. Starch, such as potato starch, is often added in the range of 8-15% to modify the textural properties of surimi-based seafoods.

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The potential of using yam as a health ingredient and an alternative source for starch in surimi seafoods was evaluated by determining the antioxidative activities and textural properties of surimi gels containing fresh yam and freeze or hot-air dried yam powders (Chiang, Chou & Hsu, 2005). Chiang et al. (2005) demonstrated a significant loss of antioxidant activity of yam in surimi gel containing 20% yam. During surimi gel preparation, salt is added to solublize fish proteins prior to a heating scheme to denature the fish proteins. The denatured fish proteins form a three-dimensional structure with unique textural properties (Lee 1984; Matsumoto & Noguchi, 1992). Therefore, factors in the surimi gel-forming process contributing to the loss of antioxidant activity of yam might include heating, salt addition and the association between fish proteins and the antioxidants in yam. The main goal of this study is to determine the factors in the surimi gel-forming process causing the loss of the antioxidant activity of yam in yam-containing surimi gel.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals and Materials

α,α̃-Diphenyl-β̃-picryl-hydrazyl (DPPH), gallic acid and trolox were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). *Dioscorea alata* Tainung No. 1 (TNG1) yam was purchased from a farmer in Mingjian Shiang, Nantou County, Taiwan, ROC. Yams were stored at 17 ± 2°C until use. Frozen high grade Alaska pollock (*Theragra chalcogramma*) surimi were purchased from Fu-Jen's Corporation (Taichung, Taiwan, ROC). The surimi was stored at -20°C until use.

### 2.2 Experimental Design

In this study, we determined heating and/or salt effects on the total phenolic content and DPPH radical scavenging activity of a 50% ethanol extract from yam, under the experimental conditions of heating at 90°C for 30 min and/or adding 2% NaCl. To determine the interaction between fish proteins in surimi and the antioxidants in yam, the total phenolic content and DPPH radical scavenging activity of the 50% ethanolic extracts from 20% yam-containing surimi samples were measured under four different process conditions: (1) without heating or with 2% NaCl, (2) heating at 90°C for 30 min without 2% NaCl, (3) adding 2% NaCl without heating, and (4) heating at 90°C for 30 min with 2% NaCl.

Another set of experiments using a pure phenolic compound, gallic acid, was also conducted to determine the possible association between fish proteins in surimi and pure phenolic compounds. The use of pure compound allowed us to evaluate the possible association quantitatively. It has been reported that the antioxidants in yam include phenolic compounds, mucilage and storage protein (dioscorin) (Hsu,

Chen, Weng & Tseng, 2003; Hou et al., 2001; Hou, Hsu & Lee, 2002). Therefore, in terms of pure compound, the use of pure phenolic compounds was the best option. Thus, the extractable gallic acid and DPPH radical scavenging activity of the 50% ethanolic extracts from gallic acid-containing surimi samples were also measured under four different process conditions: (1) without heating or with 2% NaCl, (2) heating at 90°C for 30 min without 2% NaCl, (3) adding 2% NaCl without heating, and (4) heating at 90°C for 30 min with 2% NaCl. Moreover, the effects of heating at 90°C for 30 min and adding 2% NaCl on the amount of free gallic acid in 50% ethanol solution and the DPPH radical scavenging activity of gallic acid were also determined.

### 2.3 Preparation of 50% Ethanolic Extracts

The yam was peeled and then cut into small chunks about 1 $cm^3$. Twenty grams of the sample was mixed with 100 ml 50% ethanol in a blender for 4 min and then the mixture was further stirred with a magnetic bar at room temperature for 60 min. After centrifuging the mixture at 2400 x g (4°C) for 10 min, the supernatant was collected for the measurement of antioxidant activity.

### 2.4 Preparation of Yam-containing and Gallic Acid-containing Surimi Samples

The frozen surimi was partially thawed at room temperature for approximately 2 hr to prevent the rapid increase of sample temperature during the subsequent blending procedures. Surimi was blended with 20% yam with or without 2% NaCl in a mixer (Model AT7, Moulinex Co., Ecully, France) for 4 min to produce a mixture with total weight of 1 kg. Ice/water was also added during mixing to adjust the final moisture content to 75% while maintaining the temperature of the mixture in the range of 5-10°C. For surimi gels containing gallic acid, gallic acid was added into ice/water mixture and then added to the surimi during mixing to allow uniform distribution.

For yam-containing or gallic acid-containing surimi samples with heating process, the blended mixture was extruded into stainless steel cooking tubes (inside diameter 3.0 cm, length 15 cm), and surimi gels were then produced by heating in a 90°C water bath for 30 min, followed by cooling in ice water for 10 min. Twenty grams of the gelled sample was extracted with 100 ml 50% ethanol as mentioned previously. For yam-containing or gallic acid-containing surimi samples without the heating process, twenty grams of the blended mixture was extracted with 100 ml 50% ethanol, as previously mentioned.

### 2.5 Determination of Total Phenolic Content

The total phenol content was analyzed using the Folin-Ciocalteu's reagent method (Sato, Ramarathnam, Suzuki, Ohkubo, Takeuchi & Ochi, 1996). Samples

were blend with 50% ethanol, and then an aliquot of the mixture (0.5 ml, 200 mg sample / ml 50% ethanol) was further mixed with 0.5 ml of Folin-Ciocalteu's reagent and 0.05 ml of 10 % $Na_2CO_3$, and the absorbance was measured at 735 nm after 1 hr incubation at room temperature. Gallic acid was used as the standard for the calibration curve, and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested dry samples.

### 2.6 Measurement of DPPH Radical-scavenging Activity

Yam's DPPH radical-scavenging activity was measured according to the method of Chung, Chang, Chao, Lin & Chou, (2002). The samples were blended with 50% ethanol, and then an aliquot of the mixture (100 μl, 200 mg sample / ml 50% ethanol) was further mixed with 100 mM Tris-HCl buffer (400 μl, pH 7.4) and then added to 1ml of 500 μM DPPH in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left in the dark at room temperature for 20 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Trolox (0.04~1.25 mg/ml) was used as the standard for the calibration curve, and the DPPH radical-scavenging activities were expressed as mole trolox equivalents per gram of tested dry samples. For the gallic acid solution and gallic acid-containing surimi samples, the DPPH radical-scavenging activities were simply expressed as mg gallic acid equivalents per ml and mg gallic acid equivalents per gram of tested dry samples, respectively.

### 2.7 Statistical Analysis

Analysis of variance (ANOVA) was conducted using a package (SAS Institute Inc., Cary, NC). A significance level of 5% was adopted for all comparisons. Duncan's multiple ranges test was used to determine the significant difference between different treatments.

## 3. RESULTS AND DISCUSSION

### 3.1 Effects of Heating and Salt on the Antioxidant Activity of Yam

The effects of heating and salt on the total phenolic content and DPPH radical scavenging activity of the 50% ethanolic extract from TNG1 are shown in Table 1. After heating at 90°C for 30 min, the total phenolic content of TNG1 decreased by about 42%, however, adding 2% NaCl showed no significant effect on the level of total phenolic content. The total phenolic content in TNG1 under the combination of heating and salt was similar to that in TNG1 with only heat treatment. Thus, our data suggested that heat treatment could cause a decrease in the total phenolic content of TNG1 but the addition of salt did not show a significant effect.

The results of DPPH radical scavenging activity were similar to those of the total phenolic content. Again, the heating scheme caused a significant decrease in the DPPH radical scavenging activity of the 50% ethanolic extract from TNG1, while adding salt did not show a significant effect in the scavenging activity. Phenolic compounds have been demonstrated to exhibit a scavenging effect for free radicals and a metal-chelating ability (Shahidi & Wanasundara, 1992). Therefore, the decline of the extractable total phenolic content due to heat treatment might be one of the factors causing the decrease in the DPPH radical scavenging activity.

Table 1. *Effects of heating (90°C, 30 min) and salt (2% NaCl) on the total phenolic content and DPPH radical scavenging activity of 50% ethanolic extract from TNG1*

| | Total phenolic content[*1] (gallic acid mg/g) | DPPH radical scavenging activity[*1] (trolox mole/g) |
|---|---|---|
| Ethanolic extract | 0.269±0.031[a] | 8.68±1.20[a] |
| Ethanolic extract with salt | 0.264±0.008[a] | 8.87±0.28[a] |
| Ethanolic extract with heating | 0.150±0.034[b] | 5.70±1.46[b] |
| Ethanolic extract with salt and heating | 0.186±0.071[ab] | 6.20±0.34[b] |

*Notes*. 1. The values are mean ± standard deviation.
2. [*1]Two way analysis of variance (ANOVA) revealed that heating was significant effect ($p<0.05$), but salt and the interaction of heating and salt were not significant ( $p > 0.05$). [a, b] Values in the same column not sharing the same superscript were significantly different from each other ($p < 0.05$).

### 3.2 Effects of the Gel-forming Process on the Antioxidant Activity of Yam-containing Surimi Gel

Once the total phenolic content and DPPH radical scavenging activity of the 50% ethanolic extract from TNG1 and pollock surimi were determined, we could calculate the estimated values for the 20% TNG1-containing surimi sample. As shown in Table 2, the total phenolic content of the 50% ethanolic extract from 20% TNG1-containing surimi sample was 0.069 mg gallic acid equivalent per gram, while the estimated value was 0.063. The results indicated that blending TNG1 with pollock surimi did not affect the extractable total phenolic content.

When TNG1 was blended with pollock surimi, the addition of 2% NaCl did not influence the total phenolic content but heating at 90°C for 30 min. did decrease the total phenolic content. The loss of phenolic content could be due to the heating itself as before-mentioned, or due to the change in the association between the fish proteins and phenolic compounds. It was possible that the association between the denatured fish proteins and phenolic compounds could affect the extractability of the phenolic compounds, even though such interaction appeared to be insignificant in the natural fish proteins. The association might be chemical interactions between the fish proteins and phenolic compounds, or simply the phenolic compounds might be trapped in the gelled network structure formed by denatured fish proteins. Our

data also showed that the combination of heating and salt further decreased the total phenolic content even though the addition of 2% NaCl alone showed no significant effect. The further decrease in the extractable total phenolic content was more likely because the addition of 2% NaCl also contributed to the change of the association. It is well known that both heating and salt are needed for fish proteins to form gelled products with high textural properties. In other words, salt can improve the gel-forming properties of surimi products. The results indicated that both heating and the association between the fish proteins and phenolic compounds could reduce the total phenolic content of the 50% ethanolic extract from the 20% TNG1-containing surimi gel.

The DPPH radical scavenging activity of the 50% ethanolic extract from TNG1-containing surimi sample without heating and salt was much lower than the estimated value, indicating the process of blending 20% TNG1 with surimi could dramatically reduce the scavenging activity (Table 2). Heating, salt and the combination of these two factors did not show a significant effect on the DPPH radical scavenging activity. Therefore, it was suggested that the natural fish proteins in pollock surimi could interact with the antioxidants in TNG1, and this interaction appeared to be the main cause for the decrease of the DPPH radical scavenging activity.

Table 2. *Effects of heating (90°C, 30 min) and salt (2% NaCl) on the total phenolic content and DPPH radical scavenging activity of 50% ethanolic extract from 20% TNG1-containing surimi gels*

| | Total phenolic content*[1] (gallic acid mg/g) | DPPH radical scavenging activity*[1] (trolox mole/g) |
|---|---|---|
| Surimi | $0.011\pm0.006^{c}$ | $0.05\pm0.05^{c}$ |
| Surimi gel without heating and salt[*2] | $0.069\pm0.016^{a}$ (0.063)[*3] | $0.30\pm.20^{ab}$ (1.78)[*3] |
| Surimi gel with salt | $0.055\pm0.009^{ab}$ | $0.51\pm0.15^{a}$ |
| Surimi gel with heating | $0.043\pm0.007^{b}$ | $0.10\pm0.14^{bc}$ |
| Surimi gel with salt and heating | $0.022\pm0.008^{c}$ | $0.18\pm0.11^{bc}$ |

*Notes*. 1. The values are mean ± standard deviation.

2. [*1] Two way analysis of variance (ANOVA) revealed that heating had a significant effect ($p<0.05$), but that salt and the interaction of heating and salt were not significant ($p>0.05$). [a, b, c] Values in the same column not sharing the same superscript were significantly different from each other ($p<0.05$). [*2] The sample was prepared by blending 20% yam and surimi without adding salt and without the subsequent heating scheme. [*3] Estimated value based on the data of yam and surimi, the calculation equation = 0.8 * the value of surimi + 0.2 * the value of yam.

### 3.3 Effects of Heating and Salt on the Antioxidant Activity of Gallic Acid

Prior to investigating the antioxidant activity of gallic acid-containing surimi gel, the effects of heating and salt on the antioxidant activity of pure gallic acid dissolved in 50% ethanol solution were determined and the results are shown in

Table 3. In the total phenolic content measurement, we used 0.03 mg/ml as the concentration for gallic acid. The results indicated that both heating at 90°C for 30 min and adding 2% NaCl did not affect the detectable amount of gallic acid in 50% ethanol solution. For the measurement of the DPPH radical scavenging activity, we selected 0.0125 mg/ml as the testing concentration for obtaining accurate spectrophometrical measurement at 517 nm. Our data indicated that the DPPH radical scavenging activity of gallic acid could be affected by both heating at 90°C for 30 min and by adding 2% NaCl. However, the combination of these two treatments did not result in further decrease in the scavenging activity.

Table 3. *Effects of heating (90°C, 30 min) and salt (2% NaCl) on the DPPH radical scavenging activity of gallic acid*

| | Gallic acid content (mg/ml) | DPPH radical scavenging activity (gallic acid mg/ml) |
|---|---|---|
| Gallic acid[*1] | 0.03[*2] | 0.0125[a] |
| Gallic acid with salt | 0.030±0.002 | 0.0070±0.0012[b] |
| Gallic acid with heating | 0.027±0.003 | 0.0071±0.0021[b] |
| Gallic acid with salt and heating | 0.031±0.004 | 0.0072±0.0011[b] |

*Notes*. 1. The values are mean ± standard deviation.
2. [a,b] Values in the same column not sharing the same superscripts were significantly different from each other ($p < 0.05$). [*1] The control represents the concentration of gallic acid used for the tests. A lower concentration was used for DPPH radical scavenging activity test to obtain a more accurate analysis. [*2] The values in the same column were not significantly different ($p > 0.05$).

### 3.4 Effects of the Gel-forming Process on the Antioxidant Activity of Gallic Acid-containing Surimi Gel

Our data indicated that an association between the denatured fish proteins in pollock surimi and the phenolic compounds in TNG1 might exist. The purpose of the set of experiment involving gallic acid was to further explore the possible association between fish proteins and pure phenolic compounds. The values of the total phenolic content and DPPH radical scavenging activity of the 50% ethanolic extract from 20% TNG1-containing surimi samples were quite low. Thus, the use of an addition of gallic acid allowed us to more accurately evaluate the effect of the gel-forming process. In gallic acid-containing surimi samples, the amount of added gallic acid was 0.3 mg/g pollock surimi (dry weight basis). As shown in Table 4, the amount of measured gallic acid in the sample was 0.28 mg/g pollock surimi, which was not statistically different from the added amount. When we compared the effects of four different treatments in the gallic acid-containing surimi samples, the data showed that the addition of 2% NaCl did not vary the gallic acid content, but the heat treatment did cause a significantly decrease. Moreover, the combination of heating and salt showed an effect which was similar to heating alone. This further indicated that it was heating and not salt causing the decline of gallic acid in the samples. However, our data also showed that gallic acid was quite heat stable in a 50% ethanol solution. We suggest that heat treatment denatures the fish proteins in pollock surimi and creates the association between the denatured

fish proteins and gallic acid, consequently decreasing the extractability of the gallic acid in the surimi gelled samples.

The measured DPPH radical scavenging activity in gallic acid-containing surimi sample without heating or salt addition was equivalent to 0.218 mg gallic acid per gram pollock surimi, which was lower than the equivalent value (0.3 mg/g) of added gallic acid (Table 4). Our data indicated that blending TNG1 with pollock surimi without heating or salt addition could reduce the DPPH radical scavenging activity of TNG1 as well as gallic acid. When comparing the effects of the four different treatments on the DPPH radical scavenging activity of the 50% ethanolic extracts from gallic acid-containing surimi samples, it was noted that heat treatment caused a significant decrease in the scavenging activity but the addition of 2% NaCl had no significant further effect.

Table 4. *Effects of heating (90°C, 30 min) and salt (2% NaCl) on the total phenolic content and DPPH radical scavenging activity of gallic acid-containing surimi gels*

| | Total phenolic content[*1] (gallic acid mg/g) | DPPH radical scavenging activity[*1] (gallic acid mg/g) |
|---|---|---|
| Gallic acid[*2] | 0.30 | 0.30 |
| Gallic acid surimi gel without salt and heating[*3] | 0.280±0.020[a] | 0.218±0.013[a] |
| Gallic acid surimi gel with salt | 0.243±0.023[a] | 0.204±0.018[a] |
| Gallic acid surimi gel with heating | 0.162±0.014[b] | 0.152±0.025[b] |
| Gallic acid surimi gel with salt and heating | 0.165±0.027[b] | 0.135±0.027[b] |

*Notes*. 1. The values are mean ± standard deviation.

2. [*1] Two way analysis of variance (ANOVA) revealed that heating had a significant effect ($p<0.05$), but salt and the interaction of heating and salt were not significant ($p>0.05$). [*2] The added amount of gallic acid in surimi gel. [*3] The sample was prepared by blending gallic acid and surimi without adding salt and without the subsequent heating scheme. [a, b] Values in the same column not sharing the same superscript were significantly different from each other ($p<0.05$).

## 4. CONCLUSIONS

Heat treatment could reduce the phenolic content and DPPH radical scavenging activity of yam. It is suggested that heat treatment may cause an interaction between the denatured fish proteins and phenolic compounds in TNG1, thus decreasing the extractability of the phenolic compounds. The addition of salt did not show a significant effect on the phenolic content and DPPH radical scavenging activity of yam. It appears that heat treatment is the main factor during surimi gel processing, which causes the decline of the phenolic content and DPPH radical scavenging activity.

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