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Evaluation of Antioxidant Activity and Fatty Acids Composition of Nile Tilapia (*Oreochromis niloticus-mossambicus*) Noodles Fortified with Riceberry Germ

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Abstract

Tilapia noodles containing tilapia fish meat as main ingredients were fortified with different concentrations of riceberry germ (0.64%, 0.98%, 1.28%). The aims of this research were to develop tilapia noodle enriched with antioxidant agent from riceberry germ, to characterize the antioxidant activity and fatty acid compositions of developed tilapia noodles fortified with riceberry germ. Oleic acids were reported to be the most abundant fatty acids found in all noodle formulation with value range from 37-42 %. High relative percentages of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were obtained from the noodle fortified with 1.28% of riceberry germ. Similarly, the highest antioxidant activity was also obtained from the noodle fortified with 1.28% of riceberry germ. The noodles enriched with high fish protein and excellent antioxidants were successfully developed in this study.

Keywords: *Antioxidant, Tilapia, Riceberry, MUFA, PUFA*

INTRODUCTION

Food products which offer extra health benefits with good sensory attributes are preferred by many consumers. The food product should not only fulfil the essential requirement but should provide functional ingredients that beneficial to our body. Fish has been long consumed by human since ancient time. It provides excellent sources of nutients like protein, vitamins, minerals, and lipids. Fish meat is suitable to all ages from children to aging individuals as it has soft texture and easy digestion and so tend to be an excellent candidate for food development. Nile tilapia is one of freshwater fish (*Oreochromis niloticus*) widely cultivated within Thailand and many parts of the world (Chen et al., 2013). This might due to the high growth rate of this fish which is good for aquaculture industry. In additions, the ability of nile tilapia fish to grow in a wide variety of environment. Thus, this nile tilapia fish become the most important aquaculture in Thailand especially the cross-breed nile tilapia (*Oreochromis niloticus-mossambicus*) by Thai CPF company. This uniqueness of fish brings high demands of both domestic and international markets. However, the coversion of this type of fish into new type of food is still needed. This is a good opportunity of the local Thai to create a new type of product derived from this Thai nile tilapia with extra health benefits. Many Thai local agricultural products have been shown to provide good functional

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ingredients. Richberry is one among local Thai agricultural product that shows to be a promising ingredient that promote food product with functional properties. Richberry are excellent sources of carotenoids, gamma-oryzanol, anthocyanins, vitamin E, lipids (Raungrusmee et al., 2022). Thus, in this study, Thai fish tilapia was used as a main ingredient for developing a high protein noodles containing functional properties derived from riceberry. The fatty acid profiles and antioxidant capacity of tilapia noodles were also investigated in this study.

LITERATURE REVIEW

2.1 Nile tilapia (Oreochromis niloticus)

Nile tilapia (*Oreochromis niloticus*) is one of significant freshwater fish in aquaculture industry. Due to a fast growing and adaptation to various environmental conditions (Chen et al., 2013). The fish meat is richs in polyunsaturated fatty acid (Omega-3), vitamins A, D and B, calcium, phosphorus, selenium and managanese (Stevanato et al., 2008). To improve the omega fatty acids in tilapia fish, many feed enriched with omega-3 and omega-6 fatty acids have been fed to the fish in the aquaculture industry. Thus, the tilapia was reported to provide the highest omega-6 contents among farm fish (Young, 2009).

2.2 Riceberry (Oryza sativa L.)

Riceberry is one of Thai rice cultivars cross breeded by the combination of Hom Nin rice (Thai non-glutinous purple rice) and Thai Hom Mali rice (Khoa Dawk Mali 105). It is a very long-grain purple rice. This Thai black rice was developed in order to provide nutritious rice with low glucose, high iron and high antioxidant contents which is good not only for normal consumer but also with the anemic and diabetes melitus patient (Chancharoonpong et al., 2021, Poosri et al., 2019, Raungrusmee et al., 2022, Suttiarporn et al., 2016). The black rice has been well-known as health promoting and superfood diet. The health benefits of black rice have been demonstrated including hypoglycemic, hypolipidemic, heapato protective, anticancer, antioxidant, anti-inflammatory activities These health attributes were derived from the phytochemical present in the riceberry which are carotenoids, gamma-oryzanol, anthocyanins, vitamin E (Raungrusmee et al., 2022, Yao et al., 2013).

RESEARCH METHOD

3.1. Materials and chemical reagents

Nile tilapia (*Oreochromis niloticus-mossambicus*) was purchased, Nakhon Nayok Province, Thailand. Riceberry germ powder was purchased from Tawan group (Bangkok, Thailand). All organic solvents were purchased from Merck (Darnstadt, Germany). Standard phenolic compounds including DPPH, Butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, USA).

3.2. Preparation of tilapia noodles fortified with riceberry

Nile tilapia fish meat, tapioca starch, riceberry germ powder, egg white, salt, sugar, white pepper, ground coriander seeds, and ice were used to prepare the noodles with the proportion of each ingredient for each formulation was shown in the Table 1. The fortified fish noodles were prepared by substituition of tapioca starch with 0.64 %, 0.98 % and 1.28 % of riceberry germ powder accordingly. In making noodles enriched with fish protein, the nile tilapia was cleaned. The nile tilapia fish was fillet and grinded using electric meat grinder. The fish meat was added in kneading machine. The ice and salts were gradually added and the mixtures were kneaded for 8 min. The other ingerdients like egg white, tapioca starch, riceberry germ powder, white pepper, sugar, ground coriander seeds were then added into kneading machine and kneaded for 10 min. The mxtures were freezed in refrigerator for 30 min. Then, the dough was molded into thin noodles. The fish noodle was then boiled at 55-66 °C for 10 min and continued boiled at 85-90 °C for 20 min. The fish noodle was soaked on ice for 10 min and was cut as needed.

Table 1. Optimization studied for 4 fish noodles with Riceberry germ powder formula.

Ingredients	**Control (g)**	**0.64 FNR (g)**	**0.98 FNR (g)**	**1.28 FNR (g)**
Nile tilapia fish meat	68.00	68.00	68.00	68.00
Tapioca starch	6.40	5.76	5.42	5.12
Riceberry germ powder	0.00	0.64	0.98	1.28
Egg white	12.00	12.00	12.00	12.00
Salt	1.40	1.40	1.40	1.40
Sugar	1.20	1.20	1.20	1.20
White pepper	0.80	0.80	0.80	0.80
Ice	10.00	10.00	10.00	10.00
Ground coriander seeds	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00

3.3. Characterization of fatty acid profiles in tilapia noodles fortified with riceberry germ by GC-FID

The fish noodle formulations containing different concentrations of riceberry were further characterized for fatty acid profiles according to (Santiworakun et al., 2022). Two grams of samples were transferred into test tubes and were extracted by dichloromethane: methanol (2:1, v/v). Then, the samples were incubated for 1 h and shaked for every 15 min. The mixture solution was then filtered by Whatman paper No. 1. KCl (0.1 mol/L) was then added and centrifuged at 2000×g for 10 min (Himac CF7D2, Hitachi, Tokyo, Japan). After centrifugation, the 200 μL of bottom phase was collected and transferred into new test tube. For fatty acid methyl esters (FAMEs) conversion, 2 mL of 0.5 M NaOH-methanol was added into the test tube and heated at 100 ◦C for 15 min and cooled down at room temperature. After that 2 mL of 14% BF_3-methanol was added into the test tube and heated at 100 ◦C for 1 min and cooled down at room temperature. 500 µL of hexane and 5 mL of saturated NaCl were added into test tube and then centrifuged at 1000×g for 5 min. The upper phase was collected and transferred into a new test tube. The fatty acid profiles of custards were analyzed by Gas chromatography (GC-FID 2010; Shimazu). The DB23 column (30 m × 0.25 mm i.d.x 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA) was used in this analysis. The helium was used as a carrier gas. The total gas flow rate was 62.9 mL/min, with split ratio 1:50. The conditions were set as follows: the initial and final column temperatures were 80 ◦C and 220 ◦C, respectively. The injector and detector temperatures were set at 250 ◦C and 300 ◦C respectively. The FAMEs standard was used for identification of fatty acid profiles in the custards. The retention times of the samples were compared with the FAMEs standard. The results were expressed as the relative percentage of each individual fatty acid.

3.4 Determination of antioxidant activity using DPPH assay

Antioxidant activity of tilapia noodles fortified with different concentrations of riceberry were measured by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Briefly, 5 g of each tilapia noodles formulation was grinded and dissolved in 25 mL of 80% ethanol solution. Then, the samples were incubated in sonicator at 40 °C for 30 min. The mixture solution was filtered with Whatman No.1 and the filtrate was kept at 4°C until further analysis. Tilapia noodles extracts (0.1 mL) and 0.208 mM DPPH dissolved in 80% ethanol solution (0.1 mL) were added. After 40 min of incubation in the dark at room temperature, the absorbance was measured against a blank (ethanol) at 515 nm (Cheng et al., 2006) using a UV/Visible spectrophotometer. Inhibition of DPPH radical was calculated as a percentage (%) using the formula:

$$\% \text{ Inhibition} = [\ (A_0 - A_1)\ A_0] x 100$$

Where A_1 = absorbance of the test sample and A_0 = absorbance of control. Each assay was carried out in triplicates. Butylated hydroxytoluene (BHT) was used as a control.

3.6. Statistical analysis

All experiments were done in triplicate and all results are presented as mean±SD. The statistical analyses of collected data were performed using SPSS version 22.0 (IBM). One-way analysis of variance (ANOVA) and multiple comparisons by Tukey's were performed to analyze the difference among data. Statistical differences were significant at P<0.05.

FINDINGS AND DISCUSSION

4.1 Characterization of fatty acid profiles in tilapia noodles fortified with riceberry germ by GC-FID

With health concerns, the fatty acid contents in food diet particularly, omega-3 and omega-6 fatty acids become important elements for promoting health benefit. Both omega-3 and omega-6 fatty acids are essentials fatty acids which could not systhesized by human body. Omega-6 fatty acids could be obtained from many sources for example egg, grains, poultry, and fish while omega-3 fatty acids are usually obtained from cold water fish. Consumption of high proportion of omega-6 than omega-3 reported to lead elevated levels of arachidonic acid and proinflammatory eicosanoids, which is a major factor in the increased incidence of circulatory and inflammatory disorders. Thus, it is recommended to consume high proportion of omega-3 than omega-6 in the diet (Young, 2009). Thus, this study is aim to develop a noodle product that contains a balance proportion of omega-3 to omega-6 according to the recommendation. According to the fatty acids profile obtained, the result indicated that the noodle fortified with riceberry germ showed to provide a better fatty acid profile compared to control sample (Table 2). As the concentration of riceberry increase, the saturated fatty acids content was decreased and the monounsaturated fatty acids content was increased. The good proportion of omega-3/6 was also found in the noodle formulations fortified with 1.28% of riceberry germ. This indicated that by alteration of the food ingredient with known fatty acid profiles, the food product with good fatty acids profiles could be promoted.

Table 2. Fatty acid compositions of riceberry germ and fish noodles fortified with riceberry germ

Compound name	Ricberry extract	Control (g)	0.64% Riceberry (g)	0.98% Riceberry (g)	1.28% Riceberry (g)
C12:0	-	0.50 ± 0.02	0.52 ± 0.02	0.54 ± 0.03	0.20 ± 0.02
C14:0	0.61 ± 0.02	3.15 ± 0.22	3.01 ± 0.11	3.21 ± 0.01	1.48 ± 0.25
C16:0	19.72 ± 0.04	28.75 ± 0.56	27.21 ± 0.29	27.52 ± 0.01	23.95 ± 0.55
C16:1 n-7	0.14 ± 0.01	3.55 ± 0.18	3.98 ± 0.19	3.59 ± 1.13	1.69 ± 0.21
C18:0	3.03 ± 0.05	6.47 ± 0.23	5.70 ± 0.40	6.22 ± 0.06	3.76 ± 0.08
C18:1n-9 cis	41.65 ± 0.06	37.00 ± 0.54	40.62 ± 0.44	40.26 ± 0.65	42.07 ± 0.28

C18:2 n-6 cis	31.23 ± 0.12	12.41 ± 0.61	11.25 ± 0.93	11.05 ± 0.72	22.92 ± 1.12
C18:3 n-3	0.99 ± 0.09	0.88 ± 0.19	0.63 ± 0.02	0.64 ± 0.07	0.22 ± 0.05
C18:3 n-6	-	0.73 ± 0.18	0.77 ± 0.01	0.78 ± 0.04	0.93 ± 0.05
C20:0	1.14 ± 0.03	-	-	-	-
C20:1 n-9	0.44 ± 0.02	1.35 ± 0.05	1.42 ± 0.06	1.50 ± 0.04	0.78 ± 0.08
C20:2	-	0.67 ± 0.05	0.65 ± 0.05	0.59 ± 0.03	0.23 ± 0.04
C20:3 n-6	-	0.58 ± 0.06	0.68 ± 0.05	0.57 ± 0.07	0.26 ± 0.03
C20:4 n-6	-	1.50 ± 0.09	1.39 ± 0.07	1.09 ± 0.05	0.54 ± 0.08
C22:0	0.39 ± 0.01	-	-	-	-
C23:0	0.07 ± 0.01	-	-	-	-
C24:0	0.59 ± 0.01	-	-	-	-
C24:1	-	0.49 ± 0.03	0.35 ± 0.04	0.56 ± 0.04	0.15 ± 0.02
C22:6 n-3	-	1.97 ± 0.07	1.83 ± 0.11	1.90 ± 0.01	0.81 ± 0.11
SFA(g/100g)	25.55	39.54	37.09	38.08	29.62
MUFA(g/100g)	42.23	42.39	46.37	45.91	44.69
PUFA(g/100g)	32.22	18.07	16.54	16.01	25.69
Omega-3 (g/100g)	0.99	2.85	2.46	2.54	1.03
Omega-6 (g/100g)	31.23	15.22	14.09	13.49	24.65
Omega-7 (g/100g)	0.14	3.55	3.98	3.59	1.69
Omega-9 (g/100g)	42.09	38.35	42.04	41.76	42.85
Omega-6/3	31.55	5.34	5.73	5.31	23.93

4.2 Determination of antioxidant activity using DPPH assay

Antioxidants are now play important role in food product. The antioxidant activity of noodles could be determined in this study via DPPH assay. The reaction was determined by UV-vis spectrophotometer at absorbance of 515 nm. In this study, noodles fortified with different concentation of riceberry (0.64%, 0.98%, 1.28%) exhibited significant dose dependent DPPH radical scavenging activity measured from 55.42 to 71.22 ($p \leq 0.05$) as shown in the Figure 1. Thus, this indicated that the hydrogen donating ability of the noodles fortified with riceberry germ to DPPH radicals. The antioxidant capacity of fortified noodles could be obtained from phenolic compound presence in the riceberry especially two major anthocyanins; cyanidin–3-O-glucoside chloride and peonidin-3-O-glucuside chloride. As these two phenolic compounds were reported to present in riceberry rice (Yao et al., 2013).

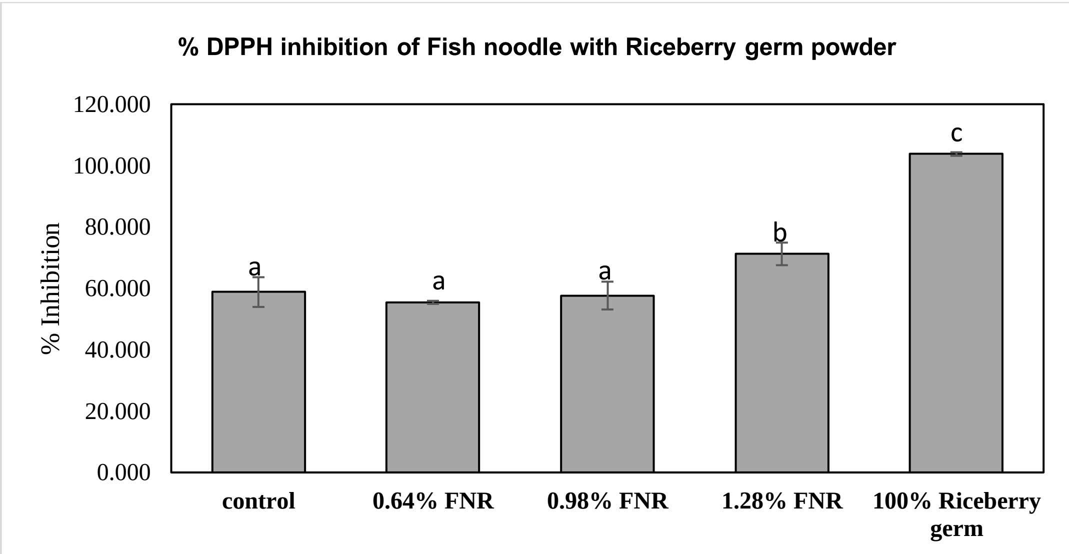

Figure 1. The antioxidant activity using DPPH scavenging assay inhibition of Fish noodle

Riceberry germ powder. Mean ± SD, the letter difference indicates statistical difference between the group at $p<0.05$ using Tukey's test

CONCLUSION AND FURTHER RESEARCH

The noodles enriched with high protein and excellent antioxidants derived from tilapia and riceberry germ were successfully developed in this study. The developed noodle provides good fatty acids profiles with high content of monounsaturated and polyunsaturated fatty acids. In additions, by DPPH assay, the result revealed that the developed noodles also contain high antioxidants. Further sensory evaluation should be investigated in order to develop a healthy food product with good sensory.

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