Incorporation of microalgae and seaweed in instant fried wheat noodles manufacturing: nutrition and culinary properties study

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Abstract
In this study, two microalgae flours (Chlorella vulgaris and Spirulina platensis), and a seaweed flour (Eucheuma cottonii) were incorporated to wheat flour in noodles manufacturing. The objective of this study was to investigate the effect of the compositions of wheat flour-marine plants flours composite and the compositions of marine plants flour composites on the nutrition contents and culinary properties of the noodles. The results showed that the incorporation of microalgae to noodles dough, increased protein, fat, ash and dietary fibre contents, but reduced carbohydrate content of the noodles. No significant change was observed for moisture content. Furthermore, the incorporation of marine plants flours to wheat flour also produced noodles with acceptable culinary properties such as, texture, colour, aroma, and flavour. The best composition of flour composite for noodle manufacturing obtained in this work was 90 g wheat flour, 5 g Spirulina platensis, and 5 g Eucheuma cottonii flours. This flour composite resulted noodle with lowest fat content but highest protein content and consumer acceptability.

Introduction
Noodles are important food products throughout the world for centuries, specifically in the Asian regions, such as China, Korea, Malaysia, Philippines and Thailand. World Instant Noodle Association (WINA) reported that noodles annual production in 2013 was 105,650 million packs with increasing rate of 3% per year since 2010 (World Instant Noodle Association, 2014). Since most noodles are made of wheat flour, increasing noodles consumption leads to massive wheat imports for non-wheat producing countries. Apart from wheat import problem, wheat consumption itself also may cause allergy, asthma, autoimmune response, or gluten sensitivity in some group of people (Rosell et al., 2014), which lead to increasing demand of low or even gluten-free products. Therefore, it is of great importance to create noodles made from non-wheat flours or wheat flour with some portion substituted with non-wheat flours. A number of research have been done on gluten-free noodle manufacturing using different types of flour, such as rice flour (Heo et al., 2014), sorghum (Liu et al., 2012), corn starch (Yousif et al., 2012) and corn flour (Padalino et al., 2013). Recently, pseudo-cereal such as amaranth flour in combination with cassava starch was also used for the making of gluten-free pasta (Fiorda et al., 2013).

Due to their sensory attributes, low cost, ease of preparation and transportation, and relatively long shelf life, noodles have been a potential vehicle for nutraceuticals, such as vitamins or polyunsaturated fatty acids (Verardo et al., 2009). In fact, noodles were also being the first foods permitted by the United States Food and Drug Administration (FDA) for vitamin and iron enrichment in the 1940s (Marconi and Carcea, 2001). Noodles and pasta have been fortified to enhance their nutritional properties with supplements from various high-protein sources, such as soy flours, soy isolates, milk and milk products, whey proteins, yeast protein concentrates and germinated pigeon pea (Torres et al., 2007). Fortification of noodles and pasta products is one of effective ways that food industry tries to answer the growing interest in functional foods that can provide physiological benefits such as having an anti-hypertensive, antioxidant or anti-inflammatory effect, in addition to the nutritional and energetic benefits (Goldberg, 1996). Consumers in most developed countries are turning to more natural and nutritional products such as marine-based products, many of which have unrivalled health properties (Netten et al., 2000).

Spirulina platensis (S.P.) grows abundantly in some alkaline lakes in Mexico and Africa, and has been utilised as a food source by local people since...
ancient times. This fact was triggered by *Spirulina*’s high protein content and excellent nutritive value, specifically the high γ-linolenic acid contents. This microalga has various health promoting effects, such as hypocholesterolaemic, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus* and anticarcinogenic activity (Spolaore *et al*., 2006). Instead of only being known as a traditional food in the Orient, *Chlorella vulgaris* (C.V) has also been used as an alternative medicine in East Asia since ancient times. *Chlorella* provides health benefits, such as assisting the healing of disorders, such as gastric ulcers, wounds, constipation, anaemia, hypertension, diabetes, infant malnutrition and neurosis (Yamaguchi, 1997). Under certain culture conditions (light stress, nutrient depletion and high salinity), *Chlorella* is able to accumulate high concentrations of carotenoids such as canthaxanthin, which may function as an antioxidant to protect against oxidation (Gouveia *et al*., 2006; Batista *et al*., 2008).

The absence of gluten in non-wheat noodle may adversely affect noodles quality. Therefore, several techniques have been used for starch textural improvement, such as annealing and hydrothermal (Hormdok and Noomhorm, 2007), gelatinisation (Yousif *et al*., 2012), fermentation (Yuan *et al*., 2008), addition of hydrocolloids such as carboxymethylcellulose (Choy *et al*., 2012), addition of konjac glucomannan (Zhou *et al*., 2013), β-glucan (Heo *et al*., 2014), addition of transglutaminase (Kim *et al*., 2014), and acetylated starch (Choy *et al*., 2012). *Eucheuma cottonii* (E.C.) or seaweed contains agar and carrageenan, which both are natural hydrocolloids (Sozer *et al*., 2007). Therefore, addition of this seaweed extracts on non-wheat flour is expected to substitute the roles of gluten in noodle manufacturing.

The objectives of this work were to study the preparation of low gluten noodles with high protein content by incorporation of marine plants flours, i.e. microalgae (*Spirulina platensis* and *Chlorella vulgaris*) and a seaweed (*Eucheuma cottonii*), and to study the nutrition and culinary properties of the noodles as affected by compositions of wheat flour-marine plants flours composite and the compositions of marine plants flour composites.

**Materials and Methods**

**Materials**

Wheat flour with “Cakra Kembar” brand, E.C. extract with “Swallow Globe” brand, eggs, and salt were obtained from local Supermarket. Microalgae S.P. and C.V. extracts powder were purchased from “Balai Besar Pengembangan Budidaya Air Payau (Centre for Development of Brackish Water Aquaculture)” Jepara, Central Java Province, Indonesia. All chemicals and reagents used were of analytical grade and purchased from Sigma-Aldrich Pte. Ltd. (Singapore).

**Noodles manufacturing**

Marine plants-wheat flours composites instant fried noodles were prepared using to the basic formulation (Moss *et al*., 1986) method with slight modification. Extract of E. C., C. V. and S. P. flours were mixed with wheat flour (W. F.) in accordance to the combination presented in Table 1. A total of 100 g of composite flour (marine plants flour–wheat flour) was weighed. Carefully weighed 12 g of the composite flour was put into a 500 mL clean beaker, mixed with 10 mL warm water (55°C), egg, 1.5% salt and 0.5% alkaline solution and then stirred completely after adding 24 mL of boiling water. The egg was properly wiped before mixing it with the constituted flour. The remaining 88 g of composite flour and 42 mL of warm water were added and stirred for 30 min at 60°C. The mixture was then kneaded with water to dough consistency and rested for 1 h in a plastic bag before sheeting. The dough were formed into dough sheets of 3 mm thickness by passing the dough through the rollers of a noodle machine (Ohtake Noodle Machine Mfg., Tokyo, Japan) at 8 rpm and a 3 mm gap; dough was folded and put through the sheeting rollers. The folding and sheeting were repeated twice. The dough sheets were rested for 30 min before further size reduction and cutting. The final cutting roll gap was adjusted to 1.0 mm and the noodle sheet was passed through a cutter and waver. The noodles were steamed in a steamer, placed into a wire basket fitted with a lid and the basket was dipped in hot palm olein at 150-160°C for 1 min, drained the palm olein and equilibrated the noodles to room temperature (30 ± 5°C) for 4 h before being packed in clean pre-sterilized polyethylene bags and stored at room temperature before further analysis.

**Nutrition and culinary properties analysis**

Starch and hydrolysable carbohydrates were determined according to the AOAC 948.02 method (AOAC, 1990). Fat was examined by weighing the dichloromethane extracts. The crude protein content was calculated by conversion of total nitrogen content (N × 6.25) obtained from digestion of noodle in a Kjeldahl apparatus following the 920.87 AOAC method (AOAC, 1990). Ash was determined by
the AOAC 923.03 method (AOAC, 1990), and the crude fibre content by the Bellucci method (Bellucci, 1932). Three grams of powder were boiled for 25 min with 50 ml of glacial acetic acid (80%, w/w) and concentrated HNO₃ solution (45/5, v/v), filtered and, after a wash with boiling water (10 mL), ethanol (20 mL) and ethyl ether (20 mL) and boiling water to neutralise, the precipitate was dried in an oven for 3 h at 105°C, weighed, burned on flame in a crucible and re-weighed. Moisture content was determined by drying of the noodles in an oven at 130°C to constant weight by the AOAC 934.06 method (AOAC, 2000).

Culinary properties analysis included sensory analysis and cooking properties analysis. Four sensorial analysis: texture, colour, odour, and flavour, were tested by 30 untrained panellists, and score values were given ranging from “don’t like” (1) to “like a lot” (10), depending on the attribute. The cooking properties which included swelling index, water absorption, and cooking loss of noodles were evaluated according to the method of Fradique et al. (2010). Ten grams of noodles was cooked in 100 mL of boiling water to obtain complete gelatinisation of starch, shown by the disappearance of the white central core, after having pressed the noodle strands between two transparent glass slides. After cooking and draining, samples were analysed for swelling index and water absorption. In addition, the cooking water was used for determination of cooking losses.

**Swelling index**

Swelling index of cooked noodles was evaluated by drying samples to constant weight at 103°C by converting the weight difference of cooked and dried noodles to volume (Brennan et al., 2004):

**Water absorption**

Water absorption of drained noodles was determined by (Bui and Small, 2007):

**Cooking loss**

Cooking loss was determined by the evaporation of the cooking water in an oven at 103°C until constant weight, according following expression (Brennan et al., 2004):

The data obtained from the studies were subjected to statistical analysis using one factor analysis of variance (ANOVA) and Turkey mean separation for multiple comparisons with the Statistical Analysis System (SAS) program (SAS Institute, 2003). Significance was accepted at P ≤ 0.05.

### Results and Discussion

#### Nutrition contents of noodles

With regard to the chemical composition of wheat, E. C., S. P. and C. V. flours (Table 1), the addition of E. C., S. P. and C. V. flours to wheat flour in noodles manufacturing was expected to improve the nutrition contents of the noodles. The combinations of wheat flour, E.C., S. P. and C. V. flours for noodles dough preparation are presented in Table 2. The carbohydrate, protein, fat, ash, dietary fibre and moisture contents of the noodles obtained from the experiments are tabulated in Table 3.

As seen in Table 3, carbohydrate content of noodles significantly (p < 0.05) decreased with the
increase of addition of C.V. and S.P. flours or with a decrease in wheat flour portion used. Sample 1 (control sample), which was produced from wheat flour only, contains the highest carbohydrate content (77.95%). The lowest carbohydrate content (56.38%) was found in sample 11, which was made of 70 g wheat flour, 5 g E. C. and 25 g C. V. This fact was in accordance with the value of carbohydrate content of the S. P. and C.V., which are lower than that of wheat flour (Table 1). Similar result was reported for noodles made of composite flour of wheat, milled and defatted soybean flours (Vijayakumar et al., 2010). In addition to the effect of blending with other flours, slightly lower carbohydrate content in noodles compared to wheat flour was likely due to the effect of processing. During the deep frying process, some carbohydrates may undergo uncatalysed hydrolysis into simpler sugar by water in the noodle at temperature higher than 100°C (Rogalinski et al., 2008). In addition, some carbohydrates also decomposed to phenolic and furan derivatives (Schacht et al., 2008). Those furan derivatives include 5-hydroxymethylfurfural and furfural (Nagamori and Funazukuri, 2004).

Table 3 also shows that the protein content of the noodles increased significantly (p < 0.05) with increasing S. P. and C.V. flours portions in the noodle flour composites. Sample 1, which merely made of wheat flour, has the lowest protein content. As expected, Sample 10 which was made from 70 g wheat flour, 5 g E. C. and 25 g S. P. flours has the highest protein content, up to 20.74%. This is because the protein content of S.P. is much higher than that of wheat flour, suggesting it to be one of important ingredients for protein rich noodles manufacturing (Becker, 2004). Proteins of microalgae are good for human body as they serve as building block, while the protein in wheat flour (gluten) serves as dough improver in noodles manufacturing. In addition to the effect of raw material blending composition, the lower value of protein content of noodles compared to S.P and C.V flours was suspected as a result of deep frying at high temperatures. By heating at temperatures over 90°C, protein is expected to undergo denaturation, dissociation to their constituent subunits, and then aggregation (Petrucelli and Anon, 1995). Successive re-association of dissociated protein subunits occurs at least partially through SH - SS exchange reactions. Shimoyamada et al. (2010) also reported rapid denaturation of soybean milk protein as affected by heating at 80°C-115°C for 2 minutes. Luckily, the noodles were completely fried in one minute, so that only a few proteins were denatured.

Tabel 3 shows that fat content of the noodles increased significantly (p < 0.05) with increasing of S. P. and C. V. flours portions in the noodle flour composites. Sample 1, which merely made of wheat flour, has the lowest fat content. In contrast to that, Sample 11 which made of 70 g wheat flour, 5 g E. C. and 25 g C. V. flours has the highest fat content, which is 9.25%. The increase of fat content in the noodles enriched with S.P. and C.V. was likely due to higher fat content of S.P. and C.V. flours than that of wheat flour. However, when compared with meat and milk, which contain 34% and 28% fat, respectively (Becker, 2004), S.P and C.V. incorporated noodles

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<th>Carbohydrate (%)±SD</th>
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<th>Fat (%)±SD</th>
<th>Ash (%)±SD</th>
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</table>
show their superior potential as healthy foods due to their lower fat content. The frying oil is easier to be drained from noodles rather than meat products because noodle has lower oil absorption capacity than meat (Hou et al., 1997; Hou, 2001).

As seen in Table 3, there was significant (p < 0.05) increased of ash content of the noodles with the increase of S.P. and C. V. addition. Sample 1 has the lowest ash content, while sample 10 has the highest ash content, up to 7.22%. The more S.P. and C. V. flours added to wheat flour during dough formation, the higher the ash content of the noodles produced. This is because the ash content of S. P. and C. V. are almost 20 times of that of wheat flour. Since ash content indicates the presence of minerals contained in the noodles, the noodles with some incorporation of microalgae shows their potential as healthy foods. *Chlorella* is rich in iron and calcium, while *Spirulina* is rich in potassium, calcium, and magnesium (Spolaore et al., 2006).

As tabulated in Table 3, crude fibre content of noodles increased significantly (p < 0.05) with increasing portion of S. P. and C. V. flours in the noodle flour composites. As expected, the control sample has the lowest crude fibre content, while Sample 10 which was manufactured from 70 g wheat flour, 5 g E. C. and 25 g S. P. flours, has the highest crude fibre content, up to 6.78%. In addition to S. P. and C. V. flours, E. C. flour also contributes the crude fibre content in noodle products. Crude fibre content in foods can be used as an index of dietary fibre levels, as is generally found in the raw fibre as 0.2 to 0.5 parts amount of dietary fibre (Pagan, 2012). Consumption of high-fibre foods increased faecal bulk and lowered both human serum cholesterol and the glycaemic index of foods (Trinidad et al., 2003). Roberta et al. (2014) suggested that sufficient intake of dietary fibre also improves bowel function and help to overcome constipation.

Addition of E. C., S. P. and C. V. flours to wheat flour did not significantly (P < 0.05) affect the moisture content of the noodle. The moisture contents of the noodle obtained in this work were within 7.01-7.89%. This is because the moisture content of the noodles is highly dependent on the water adsorption capacity of the flour composite and frying process during noodle manufacturing. Moisture loss is very important because the evaporation of water during the deep-frying process produces a specific structure in the instant noodles, since frying is a very fast drying process. When noodles are immersed in the hot oil (135-160°C), water vaporises very quickly from the noodles surface. Dehydration of the external surface causes water to migrate from the interior to the exterior of the noodle strands, resulting in a porous spongy structure (Hou, 2001).

### Culinary properties analysis

From experimental investigation, it was found that the optimum cooking time for all samples was 3 minutes. After 3 minutes cooking, the noodles have been completely gelatinised. The cooking quality of noodles which includes swelling index, water absorption, and cooking loss, are presented in Table 4.

It can be seen in Table 4 that there was no significant (p < 0.05) difference of swelling index of the noodles manufactured from whole wheat flour with varying S.P. and C.V. flours during dough formation.
and E. C., S. P. and C. V flours incorporated wheat flour. This is an agreement with the closely similar moisture content of all noodles, which also shows similar water holding capacity of polysaccharides in all composite flours during dough formation (Chang and Wu, 2008).

Addition of E. C., S. P. and C. V flours to wheat flour during noodle dough formation caused significant (p < 0.05) decrease of water absorption of the noodles. This fact revealed that wheat flour has higher ability to associate with water under condition where water is limited, which mostly attributed by the presence of higher amount of carbohydrates (starch) and fibre in this flour (Chandra and Shamser, 2013). Therefore, addition those microalgae and seaweed flours with lower carbohydrate contents decreased the water absorption of noodles.

Cooking loss occurred as gelatinised starches dissolve and release from the surface of noodle to cooking water. Therefore, cooking loss is an important indicator of the overall noodle cooking performance by both consumers and industry (Brennan et al., 2004). The incorporation of E. C., S. P. and C. V flours in noodle manufacturing does not change noodle cooking losses significantly (p < 0.05), showing values varied around 2.13-3.01%. Considering that cooking loss values lower than 7-8% are expected for semolina spaghettis (Doxastakis et al., 2007), the marine plants incorporated noodles elaborated in this study can be regarded as high-quality instant fried noodles.

Sensory analysis of noodles is an important factor to their possible future commercialisation, since it gives a perspective of the potential consumer’s acceptance. The results of sensory analysis the noodles obtained in this work, which consist of texture, colour, odour, and flavour are presented in Table 5. All sensory parameters for samples with 10% and 20% wheat flour substitution with marine plant flours, scored positively (>6) revealing a good acceptance of the developed products by the panellists. On the other hand, noodles obtained from composite flours with 30% wheat flour substitution with marine plant flours, scored diversely (4.70-7.07) showing a little acceptance of the developed products by the panellists.

Increasing wheat flour substitution with E. C., S. P. and C. V. flours cause reduction of noodles texture as indicated by some breakage of noodles after cooking. Most panellists preferred noodles with 10% wheat flour substitution with E. C., S. P. and C. V. flours because of interesting light green colour of the noodles. However, too much substitution of wheat flour substitution with E. C., S. P. and C. V. flours resulted in dark green noodles, which most panellists did not like. Similar to colour appreciation, panellists also preferred noodles manufactured from flour composites with 10% wheat flour substitution with E. C., S. P. and C. V. flours because of acceptable aroma of the noodles. Higher substitution percentage of wheat flour with E. C., S. P. and C. V. flours strengthen the fishy aroma. Little addition of E. C.,

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<td>16</td>
<td>5.47±0.12</td>
<td>6.83±0.10</td>
<td>4.97±0.09</td>
<td>5.63±0.05</td>
</tr>
</tbody>
</table>
S. P. and C. V. flours to wheat flour resulted delicious noodles with unique flavour. As shown in Table 5, panellists expressed their good acceptance to noodles manufactured from flour composites with 10% wheat flour substitution with E. C., S. P. and C. V. flours. Overall, the assessment of the panellists showed noodles with the addition of marine plant flours can be used as a new innovation of noodle products.

Conclusions

Addition of marine plants (E. C., S. P. and C.V) flours in the manufacture of noodles increased protein, fat, ash, and dietary fibre contents. In contrast to that, this effort reduced carbohydrate contents of the noodles. No significant changes were found for moisture content. The noodles obtained from flour composite with 10% wheat flour substitution with E. C., S. P. and C. V. flours have acceptable culinary properties in term of texture, colour, odour, and flavour. The best composition of flour composite for noodle manufacturing obtained in this work was 90 g wheat flour, 5 g *Spirulina platensis*, and 5 g *Eucheuma cottonii* flours. This flour composite resulted noodle with lowest fat content but highest protein content and consumer acceptability.

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References


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