

## Product development of Wolffia-pork ball

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### Abstract

This study investigated the use of sun-dried *Wolffia* (*Wolffia arrhiza*) as a substitute of pork in a pork ball product. Qualitative descriptive analysis and ideal ratio profile test were used to outline product's characteristics and evaluate recipe trials by 15 trained panellists. Upon agreed quality attributes, levels of *Wolffia* and spice (mixed garlic and pepper, 1:1 weight basis) in the product were optimised. Randomised complete block design was used to compare sample-to-ideal (S/I) score ratios to ideal-to-ideal (I/I) score ratios. It was found that the optimised product had a moisture content of  $76.82 \pm 0.23\%$ , pH value of  $6.05 \pm 0.01$ , and colour values (L, a, and b) of  $59.53 \pm 0.06$ ,  $-2.28 \pm 0.13$ , and  $16.06 \pm 0.09$ , respectively. Microbiological analysis results showed total plate count number of  $2.00 \times 10^2$  CFU/g, and yeast and mould count of  $< 10$  CFU/g. The developed product was subsequently evaluated on 7-point hedonic scale by 300 general consumers, in Muang, Chiangkan, and Phakao districts in Loei province, Thailand. It was found that the product got highest percentages on 'most liking' ratings on colour, spice odour, firmness, sandiness, taste and overall liking.

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### Introduction

*Wolffia* (*Wolffia arrhiza*) or watermeal is considered the smallest flowering aquatic plant. It is oval or round in shape with less than 1.5 mm in diameter, and is green or yellow-green in colour (Lynch Jr, 2004). It is a native plant that has long been used as a protein source in the north and north-eastern regions of Thailand, Burma, and Lao PDR for many generations (Bhanthumnavin and McGarry, 1971). The plant can be cultivated and is edible from November to July (Edwards, 1980). *Wolffia* has been reported to be nutritious since it has high levels of calcium (59 mg/100g), phosphorus (25 mg/100g) iron (6.6 mg/100g), and  $\beta$ -carotene (65 RE/100g) (Kriengsuwan, 2006). In the USA, *Wolffia* is sometimes regarded as weed (Lembi, 2002; Lynch Jr., 2004). However, it is also used as food ingredient in many western dishes, such as *Wolffia* muffins, *Wolffia*-tomato sandwiches, *Wolffia* dip, and *Wolffia* pies (Armstrong, 2001).

Regarding the use of *Wolffia* in Thailand, apart from being used for animal food (Charoentesprasit and Jiwyam, 2001; Chantiratikul and Chumpawadee, 2011), it has been generally used in savoury local

northern and north-eastern dishes. Recent survey conducted by our research group on *Wolffia* consumption in Loei province, Thailand, showed that the locals still consume *Wolffia* in traditional ways, i.e. as an ingredient in spicy soup and spicy salad. The survey also indicated that there was a high demand of *Wolffia* incorporated in other local foods, especially *Wolffia*-pork ball and fermented *Wolffia*-pork sausage. Therefore, this study aimed to develop *Wolffia*-pork ball as an alternative choice for *Wolffia* consumption.

### Materials and Methods

*Wolffia*, pork, and all food ingredients were purchased from local markets in Loei province, Thailand. All chemicals were obtained from BDH (Bangkok, Thailand) unless otherwise stated. Culture media were purchased from HiMedia (Bangkok, Thailand).

#### *Wolffia* preparation

Our previous study (unpublished data) on the sun drying process showed that, after cleaning with potable water and draining through a cheese cloth

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for 10 min, sun drying ( $40 \pm 5^\circ\text{C}$ ) for 7 h was an appropriate method to dry *Wolffia*. The sun-dried *Wolffia* had 3.25% moisture, 7,284.44  $\mu\text{g}/100\text{g}$   $\beta$ -carotene,  $a_w$  of 0.56, reconstitution coefficient of 0.39, and colour values (L, a, b) of 28.16, -4.81, and 23.67, respectively. The sun-dried *Wolffia* was stored in vacuum-sealed bags (250 g) in a refrigerator ( $4 \pm 1^\circ\text{C}$ ). The sun-dried *Wolffia* was rehydrated by soaking in potable water for 15 min and draining before use.

#### *Study on the characteristics outline of the product*

Trials on developing a *Wolffia*-pork ball product were performed. A typical recipe for pork ball (74.41% pork, 14.66% ice, 3.00% spice (mixed garlic and pepper, 1:1 weight basis), 3.66% glutinous rice flour, 1.59% salt, 1.45% sugar, 0.58% soy sauce, 0.36% monosodium glutamate (MSG), and 0.29% sodium triphosphate) was used as base for product development. Sun-dried *Wolffia* was used to substitute pork at 3 ratios (12.50:87.50, 18.75:81.25, and 25.00:75.00). Prior to sample assessments, 15 trained panelists were recruited and qualitative descriptive analysis (Lawless and Heyman, 1999) was performed to make agreement upon appropriate quality attributes for the product. Those attributes included colour, spice odour, *Wolffia* (savory) odour, firmness, sandiness, taste, and overall liking. Ideal ratio profile test (Sintawalai, 1998) was performed to obtain the characteristics outline of the product, which was subsequently used as reference points for recipe optimisation. The experiment was performed in triplicate. Analysis of variance with randomised complete block design (RCBD) was used in statistical analysis. Differences between mean values of the treatments were subsequently analysed by Fisher's least significant difference (LSD;  $\alpha = 0.05$ ; O'Mahony, 1985).

#### *Recipe optimisation*

Ideal ratio profile tests of the aforementioned trials showed that panelists preferred products containing *Wolffia*-to-pork ratios between 18.75 and 25% and higher amount of spice. Therefore, the recipe was optimised into 4 treatments by varying 2 levels of *Wolffia*-to-pork ratios and 2 levels of spice (mixed garlic and pepper, 1:1 weight basis), while the percentages of other ingredients remained the same as in previous trials (Table 1). Ratio profile test of each attribute and analysis of variance among sample means were subsequently carried out in the same fashion as described earlier. The mean difference between S/I and I/I ratio of each attribute was analysed using t-test and displayed on a spider chart.

Recipe optimisation was continued until there was no statistical difference between the mean values of S/I and I/I ratio of all attribute. The experiment was performed in triplicate.

#### *Physical, chemical, and microbiological analysis*

The samples were subsequently subjected to measurements of moisture content (AOAC, 1995), pH, and colour values (MiniScan XE, Hunter Lab, Thailand). For the most preferred sample, proximate analysis,  $\beta$ -carotene content, total plate count, and yeast and mould count (AOAC, 1995) were also determined. All tests were performed in triplicate.

#### *Sensory evaluation by general consumers*

The most preferred sample derived from Table 1 was evaluated by general (untrained) consumers in three districts (Muang, Chiankan, and Phakao) in Loei province, Thailand. One hundred locals and tourists in each location were asked to rate the product on the aforementioned quality attributes, using a 7-point hedonic scale (7 = extremely like, 1 = extremely dislike). Percentages of ranked scores on each attribute were calculated.

## **Results and Discussion**

#### *Study on the characteristics outline of the product*

A survey on desirable *Wolffia* products was conducted using a questionnaire (unpublished data). From 300 responses, it was found that *Wolffia*-pork ball was the highest demanded product. Other suggested products included *Wolffia*-pork fermented sausage, *Wolffia*-rice noodle, *Wolffia* crisps, *Wolffia* cookies, and *Wolffia* bread. Based on the result of the survey, therefore, development of *Wolffia*-pork ball was selected. The characteristics outline of *Wolffia*-pork balls was discussed by 15 trained panelists in qualitative descriptive analysis sessions. The sun-dried *Wolffia* had quite intense dark green-yellow colour (L, a, b values of 28.16, -4.81, and 23.67, respectively). This was because chlorophylls' structures were destroyed and/or transformed into pheophytins by heat treatment, resulting in darker green-yellow colour (Coulter, 1996). Simultaneously, the yellow colour of carotenoids, which was previously blocked by chlorophylls, became more dominant (Rocha *et al.*, 1993). Therefore, the final product was expected to have dark green-yellow colour, *Wolffia* odour, and sandiness mouthfeel. Three *Wolffia*-pork ball samples, containing different *Wolffia*-to-pork ratios, were evaluated by the same panel. It was found that the levels of *Wolffia* addition affected quality attributes of the samples (Figure 1). Darker sample colour, stronger *Wolffia* odour, and weaker spice odour were

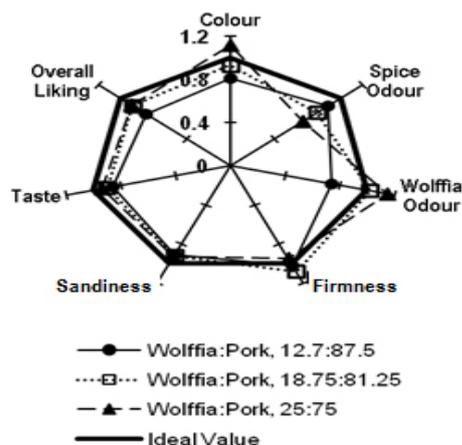


Figure 1. Comparison of ideal-to-ideal score ratio (I/I) and sample-to-ideal score ratio (S/I) of Wolffia-pork ball samples at different Wolffia-to-pork ratios on each attribute

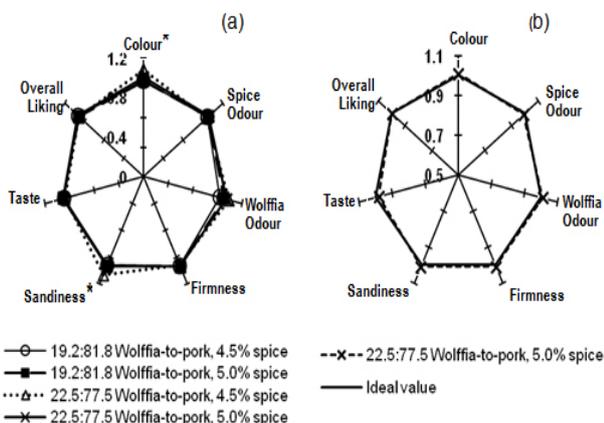


Figure 2. Comparison of (a) sample-to-ideal score ratios (S/I) of Wolffia-pork balls samples with different levels of Wolffia and spice, and (b) sample-to-ideal score ratios (S/I) and ideal-to-ideal score ratio (I/I) of the optimised Wolffia-pork ball product on each attribute. Attribute with an asterisk indicated significant difference at least between one pair of samples ( $p < 0.05$ )

detected with higher amount of Wolffia added to the sample ( $p < 0.05$ ). Although not significant ( $p > 0.05$ ), samples with higher levels of Wolffia tended to be less firm in texture. This could be because there was less protein binding capacity from pork, which was replaced by Wolffia, to form emulsion (Devatkal *et al.*, 2004). Panelists could not pick up differences between the three samples in terms of sandiness and taste ( $p > 0.05$ ). The overall liking result showed that samples with Wolffia-to-pork ratios of 18.75:81.25 and 25:75 were more preferable compared to that of 12.5:87.5 ( $p < 0.05$ ). Nevertheless, at those levels the smell of Wolffia was strong and overpowered the spice odour. Therefore, level of spices and Wolffia-to-pork ratios were optimised in the next stage.

*Recipe optimisation*

The Wolffia-pork ball recipe was adjusted by producing four Wolffia-pork ball samples with 2

Table 1. Ingredient optimisation for Wolffia-pork ball development

Ingredient	Percentage of total mix (by weight)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Wolffia-to-pork ratio	19.2:80.8	19.2:80.8	22.5:77.5	22.5:77.5
Spice <sup>1</sup>	4.5	5.0	4.5	5.0

<sup>1</sup> Mixed garlic and pepper (1:1 weight basis)

Table 2. Basic physical and chemical determination<sup>1</sup> of Wolffia-pork ball samples with different levels of Wolffia-to-pork ratios and spice<sup>2</sup>

Wolffia:Pork	Spice level (%)	Moisture (%)	pH	Colour Value		
				L	a	b
19.2:80.8	4.50	76.84±0.10	6.05±0.00	60.20±0.09	-1.64±0.24	16.12±0.14
19.2:80.8	5.00	76.29±0.15	6.05±0.01	60.21±0.03	-1.40±0.08	16.99±0.04
22.5:77.5	4.50	76.01±0.23	6.04±0.01	59.08±0.06	-2.30±0.13	16.09±0.18
22.5:77.5	5.00	76.82±0.23	6.05±0.01	59.53±0.06	-2.28±0.13	16.06±0.09

<sup>1</sup> Values were mean ± standard deviation of triplicate determinations.

<sup>2</sup> Mixed garlic and pepper (1:1 weight basis)

<sup>a,b</sup> Means with different superscript letters within each column differed significantly ( $p < 0.05$ )

different levels of spice (4.5 and 5%) and Wolffia-to-pork ratios (19.2:80.8 and 22.5:77.5; Table 1). The levels of Wolffia and spice addition did not affect the values of moisture content and pH of those four samples ( $p > 0.05$ ; Table 2). Addition of high level of Wolffia (22.5:77.5 Wolffia-to-pork ratio) resulted in significant decrease in lightness (L value) and increase in greenness of the samples ( $p < 0.05$ ). Sensory evaluation results also showed that panelists detected stronger Wolffia odour and more sandy texture in samples with high level of Wolffia addition ( $p < 0.05$ , Figure 2(a)). Regarding the spice odour, it was found that, the increased amount of spice added (4.5% and 5% w/w), resulted in better acceptance compared to the previous trials (3% w/w spice). Sensory evaluation showed non-significant differences between S/I and I/I score ratios on spice odour ( $p > 0.05$ ), which means the panelists perceived it as ideal. This was in accordance with a previous study on Wiener type sausage, reporting that addition of spices in the sausage improved panelists' odour perception by masking off-odour volatile compounds (Kryzstofiak, 2005).

The S/I score ratios all quality attributes of each sample were compared with I/I score ratios. It was found that, out of the four samples, only the sample containing 22.50:77.50 Wolffia-to-pork and 5.0% spices did not differ from the ideal value on all agreed quality attributes ( $p > 0.05$ , Figure 2(b)), which means such sample fully satisfied the panelists' expectations. Therefore, such sample was considered the most preferred product.

Table 3. Chemical and microbiological analysis<sup>1</sup> of Wolffia-pork ball in comparison with the Thai community product standard for pork ball (TISI, 2004)

Parameter	Wolffia-pork ball product	Thai community product standard for pork ball (TCPS304/2547)
Protein (%)	14.63 ± 0.30	≥ 14.00
Fat (%)	0.44 ± 0.42	≤ 6.00
Total Plate Count (CFU/g)	2.00 × 10 <sup>2</sup>	≤ 1.00 × 10 <sup>4</sup>
Yeast and Mould (CFU/g)	< 10	Not Specified

<sup>1</sup>Values were mean ± standard deviation of triplicate measurements

Table 4. Percentages of scores<sup>1</sup> ranked by 300 consumers on quality attributes of the developed Wolffia-pork ball product

Attribute	Percentage of Score						
	1	2	3	4	5	6	7
Colour	0	1	1	1	10	31	<b>56</b>
Spice Odour	0	1	1	3	13	23	<b>59</b>
Wolffia Odour	0	0	2	5	14	31	<b>48</b>
Firmness	0	1	1	3	5	17	<b>73</b>
Sandiness	0	2	3	5	12	23	<b>55</b>
Taste	0	1	1	3	5	18	<b>72</b>
Overall Liking	0	1	1	0	6	22	<b>70</b>

<sup>1</sup>The scores 1-7 in the questionnaire represented the following feelings: 1 = extremely dislike, 2 = dislike, 3 = slightly dislike, 4 = neither like or dislike, 5 = slightly like, 6 = like, and 7 = extremely like

### Chemical and microbiological analysis

Table 3 shows chemical and microbiological analysis of the developed product in comparison with the Thai community product standard for pork ball (TCPS304/2547; TISI, 2004). It was found that the product complied with the requirements of protein and fat contents, and had total microbial count within the limits specified in the safety standards.

### Sensory evaluation by general consumers

Market trials of the developed Wolffia-pork ball product was performed. Three hundreds general consumers in 3 districts of Loei province, were asked to test the product and rated the product on 7-point hedonic scale (1 = extremely dislike, and 7 = extremely like). It was found that 83% of the consumers regularly consumed meat products (sausages, fermented sausages, meat balls). Among these, 80% considered meat-vegetable mixed products, such as seaweed-fish balls, vegetable-pork balls, were healthier than meat-only ball product. Factors influencing them to decide whether to buy food products available on the market were (1) nutrition values, (2) taste, (3) price, (4) convenience, and (5) appearance. It was found

that the consumers were satisfied with the product because more than 75% of the consumers rated the product at scores of 6 (like) and 7 (extremely like) on all quality attributes tested (Table 4).

### Conclusions

In this study, Wolffia-pork ball product was developed. Recipe optimisation showed that the desirable Wolffia-pork ball product composed of 56.23% pork, 16.32% Wolffia, 14.52% ice, 5.00% mixed garlic and pepper (1:1 weight basis), 3.66% glutinous rice flour, 1.59% salt, 1.45% sugar, 0.58% soy sauce, 0.36% monosodium glutamate, and 0.29% sodium triphosphate. The developed product complied with the Thai standard specifications for pork ball product in terms of protein and fat contents as well as microbiological safety standards. The developed product was well accepted by general consumers in market trials in Loei province.

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