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Research Article

Development of fermented oyster-mushroom sausage

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Abstract

This project addressed the consumer driven need for the development of a healthier substitute to the Thai fermented pork sausage; a traditional popular product which, however, contains very high levels of saturated fat. Oyster mushrooms were used as a substitute for pork and the optimal ratio of mushroom to rice was revealed through a two-stage recipe optimisation process. Using this ratio, a trial was conducted assessing the use of different types of rice and their effect on the characteristics of the product. The final product was characterised by proximate and microbiological analysis and sensory evaluation was carried out. In conclusion, the production of an alternative to the traditional fermented Thai sausage is possible using the more economical and healthier oyster mushroom. The final product, containing a mushroom to glutinous rice ratio of 40:60, was acceptable to panellists and compared favourably against traditional fermented sausages in terms of calorific values and microbial standards.

Keywords: Thai fermented sausage, oyster mushroom, product development

Introduction

Thai fermented pork sausage is one of the most popular traditional dishes consumed nationwide in Thailand. The sausage mix contains coarsely ground pork off-cuts, coarsely ground lard, cooked rice and spices, stuffed in pork intestine or other casings [1]. After stuffing, the sausage is left to ferment at room temperature in a dry place with good ventilation for 2-3 days until it gets acidified by lactic acid producers such as *Pediococci* and *Lactobacilli* derived from the raw materials [2]. After fermentation, the pH of the sausage will be lowered to 4.8-5.3, therefore preventing the growth of other microorganisms [3]. The flavour of the sausage is derived from microbial metabolism of carbohydrates, proteins and lipids, as well as the addition of spices [4].

Oyster mushroom (*Pleurotus ostreatus*) is a common edible mushroom found in many countries in North America, Europe and Asia [5]. The shape of its cap resembles an oyster with a diameter range of 5-25cm. Oyster mushroom is considered a health food since previous studies reported it is highly nutritional, containing 1-4% protein, 0.1-0.4% fat, 0.6-2.4% fibre, 0.4-.09% ash and 89-92% moisture [6, 7, 8, 9]. Some other benefits of oyster mushroom include containing low sodium and cholesterol levels, being of low calorific value, as well as being a potential source of prebiotics [10].

In Thailand, mushroom production in 2007 was estimated at 10,000 tonnes [11]. Oyster mushroom is a widely cultivated variety commonly used in cooking. It is available throughout the year and it is among the lowest priced mushrooms in the market [12].

There have been some studies on Thai fermented pork sausage, focusing on starter culture [2], sausage quality [13], and formulation and fermentation time [14]. However, typically the product contains a high amount of saturated fat (up to 30%) [1]. This project aimed to utilise a healthy economical ingredient such as the oyster mushroom to develop a healthier alternative to the traditional fermented sausage product.

Materials and Methods

Materials

Grey-type oyster mushroom (*Pleurotus ostreatus* (Fr.) Kummer) was purchased from local markets in Muang District, Loei Province, Thailand. Fully-grown mushrooms with cap diameters between 9 and 11 cm were used throughout the experiment. All chemicals were obtained from Merck (Bangkok, Thailand) unless otherwise stated. Culture media were purchased from HiMedia (Mumbai, India).

Optimisation of oyster mushroom to cooked rice ratio

Fermented oyster mushroom sausages were produced based on a typical fermented pork sausage recipe [15]. The recipe was modified using oyster mushrooms and vegetable oil to replace minced pork and lard, respectively. Five oyster-mushroom-to-cooked-rice ratios were studied (Table 1). Oyster mushrooms were prepared by rinsing with tap water, chopping coarsely and steaming at 100ºC for 20 min to eliminate bitter taste. Excess water in the mushrooms was removed by centrifugation at 700 rpm for 5 min.

Table 1. First stage optimisation of oyster mushroom to cooked rice ratios.

Oyster mushroom: cooked rice	10:90	30:70	50:50	70:30	90:10
Oyster mushroom (%)	8.3	24.9	41.5	58.1	74.7
Cooked rice (%)	74.7	58.1	41.5	24.9	8.3

Rice was cooked in a 1.8L rice cooker. The mushroom and rice were subsequently hand-mixed with other ingredients (5% vegetable oil, 8% finely chopped garlic, 1.5% ground pepper, 1.5% sugar and 1% salt), transferred into a collagen casing (2.6cm diameter, Nippi Incorporated, Japan) and tied into 3-inch long sausages. All samples were left to ferment at ambient temperature. Samples were taken every 6h to check titratable acidity (as lactic acid) and pH [16]. The samples were kept at -18ºC to stop fermentation when pH reached 5.0±0.2. Colour measurement of the samples was performed (at RT) using a spectrophotometer (Miniscan XE, HunterLab, VA, USA). Samples were cooked and subjected to sensory evaluation and statistical analysis as described below. The most preferred treatment was selected for the second stage of optimisation. Based on the oyster-mushroom-to-cooked-rice ratio of the selected treatment, three sausage samples were prepared in which the proportion of oyster mushroom varied at ± 10% of that of the selected sample. Sausages were tested in the same manner as described earlier. The most preferred sample was selected for the next step of recipe optimisation.

Optimisation of type of rice

Six samples of sausages were produced based on the most accepted oyster-mushroom-to-cooked-rice ratio, using five varieties of rice (jasmine, brown jasmine, glutinous, black glutinous and Japanese rice) and one variety of wheat. Fermentation followed and the samples were tested in the same fashion as described above.

Sensory evaluation and statistical analysis

Sausage samples from each stage of optimisation were cooked on an electrical grill at 180-200ºC for 15 min and evaluated by 30 panellists using a 7-point hedonic scale sensory test on the following attributes – colour, odour, taste, firmness, appearance and overall liking. A score of 1 was most unacceptable while a score of 7 indicated a most acceptable sample. All tests were performed in triplicate. Analysis of variance with randomised complete block design was used in statistical analysis. Differences between mean values of the treatments were subsequently analysed by Fisher's least significant difference. SPSS software was used for the analyses.

Proximate and microbiological analysis

Microbiological safety of the product, including yeast and mould count, *Salmonella sp*. *Staphylococcus aureus*, *Clostridium perfringens* and *Escherichia coli*, was evaluated [17]. Calorific values of the most accepted fermented oyster mushroom sausage and three local-made fermented pork samples were calculated and compared based on their compositions analysed by proximate analysis [16]. All tests were performed in triplicate.

Results and Discussion

Fermented oyster mushroom sausage was produced based on a typical recipe of fermented pork sausage. Preliminary study showed that using fresh mushroom caused bitterness in the finished product. This could be due to some peptides or proteins containing bitter amino acids – such as arginine, histamine, isoleucine, leucine, methionine, phenylalanine, trypsine and valine – present in the mushroom. Previous studies reported oyster mushroom contained free bitter amino acids varying from 1-6 mg/g dry basis [9, 18, 19]. There were reports that soaking mushrooms in brine or in vinegar could reduce bitterness [20, 21]. However, those methods could affect the taste of the final product. Therefore, trials were performed and it was found that bitterness reduction could also be achieved by cooking coarsely chopped mushroom (steaming at 100ºC for 20min) and then centrifugation (700 rpm, 5 min) to remove excess water. This could be because of the protein denaturation by heat [22]. Fresh oyster mushroom prepared through this protocol gave 60% (w/w) yield and had a moisture content of 76.96±0.65%. The cooked rice gained 195% (w/w) of its original weight during preparation and had a moisture content of 68.82±1.05%

Optimisation of oyster mushroom to cooked rice ratio – first stage

Five fermented oyster mushroom sausage samples were prepared according to proportions of oyster mushroom to cooked rice shown in Table 1. Lactic acid contents and pH of the samples were monitored every 6h. The results (Figure 1a) showed that fermentation was faster in the samples containing more oyster mushroom. This could be due to difference in moisture contents between samples. As the prepared mushroom had higher moisture content than that of cooked rice, samples containing more mushroom had higher moisture content and

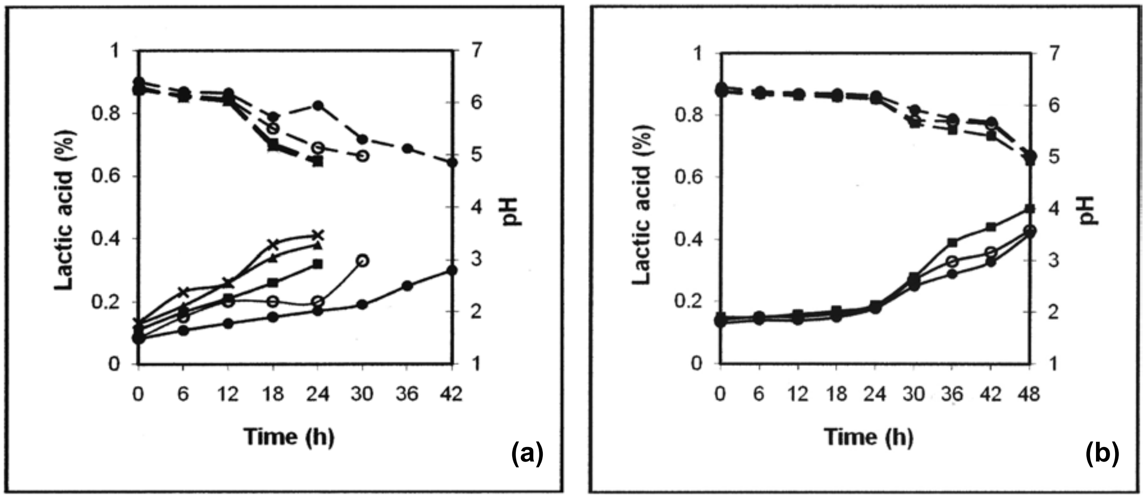

Figure 1. Relationships between pH and fermentation time (dash line) and lactic acid content and fermentation time (line), at room temperature in sausages with different proportion of oyster mushroom and cooked rice.
(a) 10:90, full circle; 30:70, open circle; 50:50, rectangle; 70:30, triangle; and 90:10, cross; and (b) 40:60, full circle; 50:50, open circle; and 60:40, rectangle.

Table 2. Colour measurement of fermented oyster mushroom sausages at different oyster-mushroom-to-cooked-rice ratios.

Oyster mushroom: cooked rice ratios in fermented sausages	Colour values*		
	L	a	b
10:90	61.24±0.50^{a}	3.20±0.25	19.21±0.26^{a}
30:70	57.72±1.83^{b}	3.06±0.27	17.47±0.28^{ab}
50:50	55.77±1.30^{bc}	2.75±0.06	16.54±0.04^{b}
70:30	53.42±3.38^{c}	2.70±0.55	15.74±0.11^{c}
90:10	53.31±0.43^{c}	3.22±0.21	16.87±0.22^{b}

* Values shown are mean value of triplicate measurements ± standard deviation. Different letters on the same column denote statistical difference between treatments according to Duncan's multiple range test at 95% confidence interval.

might be more suitable for the growth of lactic acid producers. This is in agreement with Troller and Stinson [23], who reported that lactic acid bacteria had a higher growth rate and produced more lactic acid in basal milk medium with higher a_w.

Regarding the colour of finished products, the more oyster mushroom the product contained, the darker the colour (Table 2). This is due to the natural colour of the mushroom, which had L, a, b values of 56.35±0.94, 1.89±0.12 and 13.97±0.08, respectively. Sensory evaluation showed panelists preferred the sample that had oyster-mushroom-to-cooked-rice ratios of 50:50 (Table 3).

Table 3. Sensory evaluation of different formulations of fermented oyster mushroom sausages (30 panelists; 7-point hedonic scale sensory test), first stage of optimization.

Oyster mushroom: cooked rice ratios	Average scores on sensory attributes*					
	Colour	Odour	Taste	Firmness	Appearance	Overall Liking
10:90	3.73^{b} ±1.54	3.56^{bc} ±1.53	2.60^{d} ±1.54	3.20^{c} ±1.88	3.66^{c} ±1.70	3.33^{c} ±1.60
30:70	4.00^{b} ±1.47	4.16^{bc} ±1.77	3.70^{c} ±1.68	3.90^{bc} ±1.92	3.83^{bc} ±1.79	4.06^{bc} ±1.51
50:50	5.64^{a} ±1.56	4.96^{a} ±1.31	4.83^{a} ±1.46	5.00^{a} ±1.56	5.06^{a} ±1.26	5.56^{a} ±1.43
70:30	5.26^{a} ±1.73	4.60^{a} ±1.63	4.53^{ab} ±1.38	4.50^{a} ±1.52	4.46^{ab} ±1.42	4.70^{b} ±1.17
90:10	3.93^{b} ±1.70	3.86^{bc} ±1.85	3.90^{bc} ±1.59	4.20^{ab} ±1.46	4.06^{bc} ±1.38	4.20^{b} ±1.5

* Values shown were mean value ± standard deviation of triplicate measurements. Different letters on the same column denote statistical difference between treatments according to Duncan's multiple range test at 95% confidence interval.

Optimisation of oyster mushroom to cooked rice ratio – second stage

Three fermented oyster mushroom sausage samples with oyster-mushroom-to-cooked-rice ratios of 40:60, 50:50, and 60:50 were prepared. Again, fermentation took place faster in samples with the higher proportion of oyster mushroom, which was in accordance with the results shown earlier (Figure 1b). Sensory evaluation showed that fermented sausage with oyster-mushroom-to-cooked-rice ratio of 40:60 was the most preferred sample (Table 4).

Table 4. Sensory evaluation of different formulations of fermented oyster mushroom sausages (30 panelists; 7-point hedonic scale sensory test), second stage of optimization.

Oyster mushroom: cooked rice ratios	Average scores on sensory attributes*					
	Colour	Odour	Taste	Firmness	Appearance	Overall Liking
40:60	5.40^{a} ±0.93	5.20^{a} ±1.28	5.30^{a} ±1.12	5.07^{a} ±1.06	5.33^{a} ±1.12	5.73^{a} ±0.74
50:50	5.20^{a} ±1.16	4.60^{ab} ±1.42	4.47^{b} ±1.35	5.17^{a} ±1.43	5.10^{ab} ±1.35	5.33^{ab} ±1.25
60:40	4.37^{b} ±1.68	4.30^{b} ±1.57	4.76^{ab} ±1.69	4.47^{b} ±1.63	4.60^{b} ±1.52	4.87^{b} ±1.48

* Values shown were mean value ± standard deviation of triplicate measurements. Different letters on the same column denote statistical difference between treatments according to Duncan's multiple range test at 95% confidence interval.

Optimisation of type of rice

Five varieties of rice and one variety of wheat were used in the production of fermented oyster mushroom sausages with oyster-mushroom-to-cooked-rice ratio of 40:60. Several strains of Lactobacilli, such as *L. plantarum*, *L. fermentum*, *L. acidophilus* and *L. amylophilus*, possessed amylolytic enzymes [24]. Previous studies reported their fermentation ability on different types of starchy materials [25, 26, 27]. Figure 2 shows that lactic acid production was slower in sausages with black glutinous and brown jasmine rice, as well as wheat. This could be because they still had the bran coat which retains the complex polysaccharides intact and therefore the bacteria could not access carbohydrates as easily as in jasmine, glutinous and Japanese rice.

Sensory evaluation showed that fermented oyster mushroom sausage with glutinous rice received the highest scores in firmness, appearance and overall liking (Table 5). This could be because glutinous rice contains more amylopectin and therefore forms stronger gels which positively affected the texture of the finished product. The sample with black glutinous rice was the most unacceptable in colour. Colour measurement showed that type of rice played an important role on the colour of finished product, especially on brightness and yellowness (L and b value, respectively, Table 6).

Table 5. Sensory evaluation of fermented oyster mushroom sausages containing different rice or wheat (30 panelists; 7-point hedonic scale sensory test).

Type of rice in fermented sausage	Average scores on sensory attributes*					
	Colour	Odour	Taste	Firmness	Appearance	Overall Liking
Glutinous rice	4.90^{ab} ±1.35	4.80^{ab} ±1,73	4.47^{a} ±1.55	5.30^{a} ±1.37	5.30^{a} ±1.23	5.27^{a} ±1.26
Black glutinous rice	3.20^{d} ±1.86	4.43^{bc} ±2.08	3.50^{d} ±2.02	3.23^{c} ±1.65	3.57^{d} ±1.85	3.63^{c} ±1.71
Jasmine rice	4.17^{bc} ±1.86	4.50^{bc} ±2.08	4.50^{ab} ±2.02	4.33^{b} ±1.65	4.17^{cde} ±1.85	5.00^{ab} ±1.71
Brown jasmine rice	4.23^{bc} ±1.61	4.75^{bc} ±1.52	4.70^{ab} ±1.60	3.53^{bcd} ±1.61	3.77^{de} ±1.43	4.43^{bc} ±1.17
Japanese rice	5.07^{a} ±1.20	4.50^{cd} ±1.72	3.73^{cd} ±1.84	4.03^{b} ±1.81	4.60^{abc} ±1.63	4.53^{b} ±1.76
Wheat	4.10^{c} ±2.02	3.97^{c} ±1.92	3.70^{cd} ±2.06	3.13^{dc} ±1.77	4.27^{bcde} ±1.64	4.13^{bc} ±1.61

* Values shown were mean value ± standard deviation of triplicate measurements. Different letters on the same column denote statistical difference between treatments according to Duncan's multiple range test at 95% confidence interval.

Calorific value and microbiological safety

Table 7 illustrates the comparison of nutritional compositions of fermented oyster mushroom sausage with mushroom to glutinous rice ratio of 40:60 and three samples of typical fermented pork sausages. The results showed that fermented oyster mushroom sausage contained more than 3 times less fat than typical fermented pork sausages and was 20-30% less calorific. Microbiological analysis (Table 8) revealed that the fermented oyster mushroom sausage was within microbial safety standard specifications for fermented pork sausage [1].

Table 6. Colour measurement of fermented oyster mushroom sausages with different types of rice or wheat at oyster-mushroom-to-cooked-rice ratios of 40:60.

Type of rice in fermented sausage	Colour values*		
	L	a	b
Glutinous rice	54.90±1.15[b]	2.36±0.22[c]	16.89±0.46[b]
Black glutinous rice	18.79±0.66[d]	4.69±0.32[ab]	1.36±0.36[c]
Jasmine rice	60.83±0.47[a]	2.24±0.20[c]	16.53±0.35[b]
Brown jasmine rice	58.65±0.07[a]	2.58±0.06[c]	17.68±0.04[b]
Japanese rice	56.16±0.63[ab]	2.58±0.01[c]	17.85±0.07[b]
Wheat	45.03±0.28[c]	6.37±0.16[a]	24.15±0.41[a]

* Values shown were mean value ± standard deviation of triplicate measurements. Different letters on the same column denote statistical difference between treatments according to Duncan's multiple range test at 95% confidence interval.

Table 7. **Comparison of nutritional compositions and calorific values of fermented oyster mushroom sausage and three samples of typical fermented pork sausages.**

Composition (%)	Fermented sausage sample			
	Oyster mushroom	Pork 1	Pork 2	Pork 3
Moisture	50.92±1.41	45.64±0.59	47.25±0.64	48.92±0.64
Protein	4.75±0.19	10.53±0.33	11.88±0.77	11.50±0.54
Fat	4.84±0.46	21.60±0.84	17.23±0.32	16.45±0.66
Ash	1.76±0.10	1.44±0.16	1.41±0.17	1.60±0.23
Fibre	0.32±0.04	0.17±0.03	0.18±0.04	0.31±0.05
Carbohydrate	41.21±1.33	23.56±0.86	25.65±1.53	24.00±0.40
Calorific value (kcal/100g)	227	331	305	290

* Values shown were mean value ± standard deviation of triplicate measurements

Table 8. **Microbiological analysis of fermented oyster mushroom sausage.**

Microorganism	Microbial count
Yeast and mould (CFU/g)	<10
Escherichia coli (MPN/g)	<3
Salmonella sp. (per 25g)	Non-detected
Staphylococcus aureus (per 0.1g)	Non-detected

* Values shown were mean value of triplicate measurements.

Conclusions

This study has revealed that the production of a healthier, vegetarian alternative to the traditional Thai fermented sausage is feasible and economical. Sensory evaluation revealed that the 40:60 ratio between oyster mushroom and glutinous rice was the most favourable formulation amongst the panellists. This formulation was found to not only meet microbiological safety standards but also to contain significantly lower levels of saturated fat, thus being healthier and contributing much less calories per serve than the traditional product.

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