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The Physicochemical and Sensorial Characteristics of Plant-based Patty Formulated with Oyster Mushroom and Rubber Seed Flour

Nur Suaidah Mohd Isa[1,2]*, Nor Alia Natasha Mahazan[1], Nor Hayati Ibrahim[1], Nurmahani Mohd Maidin[1,2], Nur Hafizah Malik[3] and Mohamad Khairi Zainol[1]

[1]Faculty of Food Science and Agrotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
[2]Food Innovation and Sustainability Research Group, Food Security Cluster, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
[3]Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Campus, 84600 Pagoh, Johor, Malaysia.

*Corresponding author:
Nur Suaidah Mohd Isa,
Faculty of Food Science and Agrotechnology,
Universiti Malaysia Terengganu,
21030 Kuala Nerus,
Terengganu,
Malaysia.
Email: n.suaidah@umt.edu.my

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Abstract

An increasing trend toward a meat-free diet is observed nowadays, as health-conscious consumers tend to limit their use of animal products due to the known association between high meat consumption and overweight, obesity, and an increased risk of chronic diseases. Rubber seed is an underutilised ingredient with a high protein content and has potential for use in the development of plant-based food products. Oyster mushrooms are low in fat and cholesterol and have an umami flavour that provides a taste similar to that of meat. Thus, this study was designed to explore the potential use of oyster mushrooms and rubber seeds as meat replacers for the development of a patty. The sensory and physicochemical features of plant-based patties with varying quantities of rubber seed flour (RSF) and oyster mushroom (OM) were determined. Sensory examination found that products containing 15% RSF were highly accepted by the panellists when compared to the other samples. The addition of RSF resulted in a substantial ($p<0.05$) increase in nutritional content, such as protein (from 2.78% to 4.54%) and crude fibre (from 1.01% to 2.11%). Furthermore, with the addition of RSF, a significant effect ($p<0.05$) on texture was observed, with increased cohesiveness and hardness. This study provides insight into the potential use of RSF for the development of plant-based products that can benefit the food industry.

INTRODUCTION

Consumers nowadays choose to limit their use of meat products in their meals. Despite the fact that meat is a significant source of high-quality dietary protein, it is high in calories and contains lipids linked to heart disease and high blood cholesterol levels [1]. Thus, meat consumption should be limited to 50 grams per day to avoid an elevated risk of non-communicable diseases [2]. Consumer awareness of the risk of overconsumption of meat products leads to an increase in plant-based diets. However, most of the plant-based food products containing meat substitutes are made from soy protein, which is known to pose the risk of allergies [3]. In addition, studies conducted by [4] reveal that most plant-based foods are low in protein. As a result, there is an increasing demand for plant-based foods made with alternative ingredients and high nutritional values. Mushrooms are high in protein, low in fat, and contain fibres, all of which are beneficial to the human body [5,6]. It also provides a meaty, umami flavour in food products. Besides that, antioxidants such as phenolic substances, flavonoids, ascorbic acid, glycosides, tocopherols, polysaccharides, ergothioneine, and carotenoids are also abundant in oyster mushrooms [7]. A previous study on a full-fat soy-based analogue meat patty found that the addition of oyster mushroom improves the overall quality of the patty, with increases in textural properties, water-holding capacity, and cooking quality [8]. In addition, studies by Wan Rosli and Solihah [9] on the nutritive quality of beef and chicken patties partially substituted with oyster mushrooms reported an increase in the protein efficiency ratio for samples containing oyster mushrooms compared with control samples without oyster mushrooms.

Besides that, incorporating oyster mushrooms into the production of fish burgers made with salmon and striped catfish by-products helps improve thawing loss and cooking yield of the frozen products [10]. Thus, the use of oyster mushrooms has been

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deemed an appropriate strategy for the substitution of meat in plant-based patties. Rubber seeds are often discarded in rubber plantations due to its low value. However, it has the potential to be used as a dietary ingredient and may have a major impact on human health due to its high concentration of vital fatty acids and minerals [11]. Furthermore, rubber seed contains high protein, making it a viable soy protein alternative in plant-based diets. The use of rubber seed flour in the making of white bread increased the ash, protein, fat, and fibre content, demonstrating its high potential as a dietary additive [11]. However, research on rubber seed flour and its use in food products is still scarce, limiting its widespread application. Thus, this study was conducted to determine the potential use of rubber seed flour in the production of plant-based patties made with oyster mushrooms.

MATERIALS AND METHODS

Materials

The ingredients required to produce a plant-based patty were oyster mushrooms, rubber seeds, sugar, salt, MSG, white pepper, garlic powder, potatoes, and white breadcrumbs. Rubber seeds were collected from a local farm at Sik, Kedah. The rubber seed shells were removed, and the seeds were cleaned and rinsed to remove any foreign material. After that, the rubber seeds were boiled for 30 mins before being dried in a 60°C drying oven until the moisture content reached 7%, which is acceptable for storage [12]. Boiling is crucial for reducing the levels of anti-nutrients and cyanogens [12,13]. Next, the rubber seed was finely chopped, milled, and sieved through a 250 µm sieve shaker and packed in a double-seal polyethylene bag. The rubber seed flour (RSF) was stored at room temperature in an airtight container for further analysis. Oyster mushrooms and other ingredients were purchased from a local market in Kuala Nerus, Terengganu, Malaysia. The oyster mushroom was cleaned, rinsed, and blanched for 30 seconds with a mushroom-to-water ratio of 1:5 (weight-to-volume) in hot water at 90°C. To keep the patties from becoming mushy, excess water was drained [14]. Then, the oyster mushroom was finely chopped, placed in a double-seal polyethylene bag, and stored in an airtight container at 5°C for 2 days prior to analysis.

Plant-based patty preparation

Plant-based patties were produced in accordance with the method described by [15]. The cleaned oyster mushroom was mixed with the obtained RSF at 95:5 (RSF5), 90:10 (RSF10), and 85:15 (RSF15) levels of substitution for plant-based patty production (**Table 1**). Rubber seed flour and oyster mushroom at a 100:0 ratio level were used as a control. The potatoes were then boiled until tender, and the dry ingredients were weighed according to the required amounts (**Table 1**). All the ingredients were mixed in a bowl chopper until homogeneous. Then, the mixture was weighed at 75 g each and shaped into patties using a patty mould. The prepared patties were then frozen by using a blast freezer at -18°C and stored at -18°C for further analysis.

Proximate analysis

Proximate analysis, including moisture, crude protein, crude fat, crude fibre, and ash content, was conducted for a patty sample incorporated with RSF, and the results were compared with those of a control sample prepared without RSF. The samples were analysed according to the technique developed by the Association of Official Analytical Chemists (AOAC) 2016 [16], with three replications (n=3), and the data were reported on a wet basis. Moisture analysis was conducted according to AOAC 934.01, while crude protein analysis was conducted according to AOAC 981.10. Fat analysis was conducted according to AOAC 954.02, crude fibre analysis was conducted according to AOAC 978.10, and ash content was conducted according to AOAC 923.03. Carbohydrate estimation was calculated with the following equation:

$$\text{Carbohydrate (\%)} = 100 - (\%\sum \text{moisture} + \text{ash} + \text{fat} + \text{protein} + \text{crude fiber})$$

Texture analysis

The texture analyser TA-XTplus (Stable Micro Systems, UK) was used for texture analysis of the samples [17]. The cooked patties were cut into cubes (2×2×0.5 cm). Using a 30 kg load cell, the hardness, cohesiveness, springiness, and chewiness of the patties were measured according to the Texture Profile Analysis (TPA) double-compression test. The texture analyser was programmed with the following parameters: pre-test speed of 1.0 mm/s, post-test speed of 2.0 mm/s, and distance of 2.0 mm.

Colour analysis

A colorimeter (Konica Minolta Chroma Meter CR-410) was used to determine the colour of cooked and uncooked patties; six patties were randomly selected for analysis. The lightness level ($L*$), redness level ($a*$), and yellowness level ($b*$) of the samples were recorded at six positions on the surface of the patties, and the average values were calculated.

Cooking properties

Six patties were randomly selected from each formulation for the determination of cooking properties. The patty was cooked for 7 minutes on each side at low heat. The percentage shrinkage of the patty was determined by using weight differences between raw and cooked patties and by measuring the diameter of the uncooked and cooked patties at three different positions as follows:

$$\text{Cooking loss determination (\%)} = \frac{Raw\ frozen\ patty\ weight\ (g) - Cooked\ patty\ weight\ (g)}{Raw\ frozen\ patty\ weight\ (g)} \times 100$$

$$\text{Reduction in patty diameter (\%)} = \frac{uncooked\ patty\ diameter - cooked\ patty\ diameter}{Uncooked\ patty\ diameter} \times 100$$

Table 1. Plant-based patty formulated with different levels of oyster mushroom (OM) and rubber seed flour (RSF).

	Control		RSF5		RSF10		RSF15	
	Weight (g)	%	Weight (g)	%	Weight (g)	%	Weight (g)	%
Oyster mushroom	480	60	440	55	400	50	360	45
Rubber seed flour	0	0	40	5	80	10	120	15
Sugar	8.44	1.05	8.44	1.05	8.44	1.05	8.44	1.05
Salt	4.00	0.5	4.00	0.5	4.00	0.5	4.00	0.5
MSG	0.56	0.07	0.56	0.07	0.56	0.07	0.56	0.07
White pepper	3.50	0.44	3.50	0.44	3.50	0.44	3.50	0.44
Garlic powder	3.50	0.44	3.50	0.44	3.50	0.44	3.50	0.44
Potato	200	25	200	25	200	25	200	25
White bread crumb	100	12.5	100	12.5	100	12.5	100	12.5
Total	800	100	800	100	800	100	800	100

Water holding capacity (WHC)

A 5-gram sample was mixed with 10 mL of water in a centrifuge tube. The centrifuged tube was vortexed for 5 minutes. The sample was then centrifuged for 30 minutes at 5000 rpm (2,795 × *g*) at room temperature [17]. The weight of the centrifuge tubes was measured after water was removed. Percentages of water holding capacity were then calculated following the equation:

$$\text{WHC}(\%) = \frac{\textit{weight of tube after decanting }(g) - \textit{weight of dry tube }(g) - \text{weight of total sample(g)}}{\textit{weight of total sample}(g)} \times 100$$

Sensory analysis

The samples were evaluated for attributes such as colour, flavour, odour, texture, taste, and overall acceptability using the 9-point hedonic scale (9 = like extremely, 8 = like very much, 7= like moderately, 6= like slightly, 5= neither nor or dislike, 4= dislike slightly, 3 dislike moderately, 2=dislike very much, 1= dislike extremely). Patty samples were cooked on a hot pan for 7 min on each side until the internal temperature reached approximately 80°C. The cooked patty samples were divided equally into 4 portions and presented to the panels in random order. The prepared samples were evaluated by 40 untrained panellists, comprised of staff and students of Universiti Malaysia Terengganu, Malaysia.

Statistical analysis

The analysis was carried out in a randomized block design with four replicates. The differences between samples were investigated using analysis of variance ANOVA), one-way ANOVA. and 2 2-sample t-test with Fisher LSD test. The differences were considered significant at p < 0.05.

RESULTS AND DISCUSSION

Sensory analysis of plant-based patty

The mean scores of sensory acceptability for patties made with different ratios of OM to RSF are presented in **Table 2**. Based on **Table 2**, it was observed that the addition of rubber seed (RSF) influences the sensorial properties of the plant-based patty, with samples containing 15% RSF (RSF15) receiving significantly higher scores for most attributes than other samples. In terms of aroma, the patty containing the highest percentage of rubber seed showed a significantly higher score (p<0.05) than the control sample. However, no significant difference (p>0.05) was observed between RSF5 and RSF10 in terms of aroma. This may be due to the favourable nutty aroma of the RSF. Furthermore, regarding colour attributes, it has been observed that higher RSF percentages are associated with higher acceptance scores. The colour of a food product is an important attribute that has the greatest influence on consumers' product choice [18]. The increased score in colour for plant-based patties made with RSF may be attributed to the browning colour of the rubber seed during the drying process that mimics the colour of meat-based patties (**Fig. 1**). A study on consumers' sensory acceptance of plant-based meat alternatives found that colour, along with blandness, was one of the attributes that led consumers to dislike a hybrid meat-mushroom burger [19]. Thus, this result indicated that the addition of RSF improves the appearance of mushroom-based patties, especially colour.

In addition, significantly higher acceptance (p<0.05) was observed for RSF15 samples compared to the control for texture, but no significant difference (p>0.05) was observed between RSF10 and RSF15 samples. However, the average texture acceptance score was 4.10±0.44 for the RSF15 sample, indicating slight dislike. This result contrasts with previous studies on mushroom-based patties and other meat-like protein alternatives, such as texturized vegetable proteins, in which a higher acceptance rate was observed compared with samples composed of 100% meat or made with non-meat-like proteins, such as pea protein [19–21]. This may be related to the lack of previous experience among panels with plant-based food products. Consumers' unfamiliarity with novel foods may lead to negative sensory perceptions and reduced overall liking of the product, as it fails to match their expectations [22]. On the other hand, higher acceptability among panels was observed for juiciness in the control sample compared with samples containing RSF, whereby an increase in RSF led to a decrease in acceptability. This may be related to a higher percentage of oyster mushrooms in the control samples with high moisture content, as reported in previous studies, in which mushroom-based patties have been shown to have higher juiciness scores [19]. The juiciness of the meat-free patties can be enhanced by adding water and plant-based fats, such as canola oil, as these have been reported to improve the juiciness of meat analogues [23].

In terms of oiliness, significantly higher acceptance (p<0.05) was observed for RSF15 compared to the other samples, while no significant difference (p>0.05) was observed between the control, RSF5, and RSF10 samples. Food composition has been reported as one of the contributing factors of oil absorption in fried foods, alongside temperature, whereby the incorporation of flour reduced uptake due to its role as a binder, as observed in the current study for patties made with RSF [24]. Moreover, the increase in RSF has led to a significant decrease (p<0.05) in the acceptability of overall taste. This is due to the bitter taste of the rubber seeds. Besides that, the addition of RSF to the formulation at higher percentages may lead to unpleasant aroma and taste, as reported by [25]. Flavour characteristics played an important role in determining final acceptance by consumers and have been identified as one of the most important attributes affecting consumers' acceptability of plant-based meat alternatives [19].

Based on overall acceptance, the control sample showed the highest score of 6.05±1.03 (slightly), followed by RSF15 with 5.62±1.02, RSF10 with 3.90±1.19, and RSF5 with an average score of 3.73±0.50. However, no significant difference (p<0.05) was observed between the average scores of the control and RSF15 samples. From the sensory evaluation, most panellists preferred the RSF15 sample compared to other samples, including the control, and significantly higher scores (p<0.05) were observed for most attributes, such as colour, aroma, texture, oiliness, and taste. These results showed that plant-based patties with a high acceptance level can be produced by incorporating rubber seed and oyster mushroom. Based on the sensory results, RSF15 has been chosen for further analysis, in which the sample was compared with a control sample containing 0% RSF in terms of physicochemical and cooking properties.

Table 2. Sensory attributes of cooked plant-based patties prepared at different ratios of OM and RSF. Control- 100:0, RSF5- 95:5, RSF10- 90:10, RSF15- 85:15.

Samples	Control	RSF5	RSF10	RSF15
Aroma	5.40±0.40[b]	4.50±0.80[c]	4.60±0.77[c]	6.00±1.08[a]
Colour	4.23±0.73[b]	4.70±0.80[b]	4.40±0.54[b]	5.40±1.02[a]
Texture	3.63±0.79[b]	3.57±0.48[b]	4.00±0.29[a]	4.10±0.44[a]
Juiciness	5.70±0.73[a]	4.93±0.62[ab]	4.63±0.57[b]	4.53±0.35[b]
Oiliness	4.37±0.16[b]	4.27±0.74[b]	4.13±0.48[b]	5.20±0.25[a]
Taste (Bitterness)	3.40±1.02[c]	3.77±1.41[bc]	4.07±0.29[ab]	4.43±0.36[a]
Taste (Overall)	5.96±1.06[a]	3.77±0.74[c]	3.90±1.19[c]	5.13±0.40[b]
Overall acceptance	6.05±1.03[a]	3.73±0.50[b]	3.93±1.02[b]	5.62±1.02[a]

Data are presented as the mean ± standard deviation (N=40). [abc] means with different letters within the same row are significantly different (p<0.05).

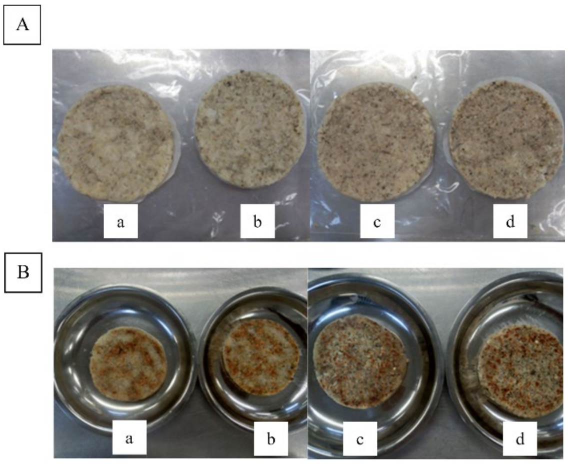

Fig. 1. Plant-based patties of uncooked (A) and cooked (B) samples prepared at different ratios of OM and RSF; a) 100:0 (Control), b) 95:5 (RSF5), c) 90:10 (RSF10), and d) 85:15 (RSF15).

Proximate analysis of plant-based patty

The moisture, ash, protein, fat, fiber, and carbohydrate contents of plant-based patties are shown in **Table 3**. It was found that patties with a 15% addition of rubber seed flour had significantly higher ash, protein, fat, and fiber content than the control. The results for moisture contents showed a significant difference ($p<0.05$) between the control and RSF15. The control showed a higher moisture content than RSF15, with values of 78.66±1.58 and 72.13±0.61, respectively. This result agrees with a study by Husain and Huda [26], which found that adding oyster mushrooms as a meat replacer in chicken nuggets increased moisture content.

The high moisture content in the control sample was related to the high moisture content in the oyster mushrooms. A previous study reported that the moisture content of oyster mushrooms was around 88.75% [27], while that of rubber seed was only 3.99% [13]. This could explain the higher moisture content in the control samples compared to those with a 15% addition of rubber seed flour. According to Sogari *et al*. [19], moisture content and the amount of liquid ingredients in patties may affect juiciness, with higher moisture content leading to greater juiciness. This is shown in the sensory results, where the control sample with the highest percentage of mushrooms had the highest juiciness score.

The plant-based patty containing 15% rubber seed flour showed a higher ash content than the control, with values of 1.53 ± 0.25 and 1.38 ± 0.28, respectively, but no significant difference ($p<0.05$) was observed between the samples. Ash content indicated the mineral content of the plant-based patties [28]. The increase in ash content may be related to the high ash content of RSF, which is consistent with a previous study reporting an increase in the percentage of ash in bread and composite flour with the addition of RSF [11]. Rubber seeds have been reported to contain high amounts of minerals. Several important minerals found in the rubber seed meal had been reported previously, including sodium, potassium, calcium, phosphorus, magnesium, iron, copper, zinc, and manganese [12]. According to Oluodo *et al*. [13], the ash content of rubber seed meal ranges from 0.2–5%, depending on genetic and environmental factors. In addition, processing methods may also affect the final ash content, whereby boiling has been shown to retain the highest amount of ash compared to toasting [12].

Thus, the lower ash content reported in the current study compared to the previous study may be attributed to the processing step, in which the seeds were boiled and dried. Based on the results, the incorporation of RSF significantly increases the amount of protein in plant-based patties, with a 63% increase observed in RSF15 compared to the control. A similar increase in protein content was also observed previously in white bread incorporated with rubber seed flour [11]. According to Udo *et al.* [12, raw rubber seed contains 23.31% crude protein, which is much higher than that of fresh oyster mushrooms, where only 3.31% crude protein was reported in the 2018 USDA Food Data Center [29]. Moreover, according to Oluodo *et al.* [13], rubber seed meal has been reported to contain higher amounts of essential and non-essential amino acids, making it a suitable alternative to soy protein in plant-based foods. However, processing rubber seeds into RSF, including boiling and drying, may lead to protein denaturation due to the high temperatures used during drying[27]. A study conducted on beef patties incorporated with corn silk reveals an increase in protein content with the addition of corn silk without affecting sensorial properties [30]. This indicates that plant-source proteins can be used to improve the nutritional qualities of patties.

The fat content of the plant-based patty was derived from the addition of the rubber seed flour. The fat content of RSF15 was significantly higher ($p<0.05$) than that of the control sample. It has been reported previously that rubber seed has a high fat content (50.2%) and is rich in oils, which comprise8.78% of the whole seed [31]. A similar increase in fat content was also observed in bread with the addition of RSF [11]. Besides protein, fat has been deemed important in the development of patties, as it may affect their texture in terms of tenderness and juiciness [32]. Furthermore, RSF15 showed a significantly higher ($p<0.05$) fibre content than the control sample, with values of 2.11 ± 0.51 and 1.01 ± 0.28, respectively (**Table 3**). An increase in fiber content was observed in a study by Husain and Huda-Faujan [26] on the use of oyster mushrooms as a meat substitute. Oyster mushroom has been reported to contain 12.87% of fibre, which is lower than that of rubber seed, which contains as high as 73.70% crude fibre [33]. The benefits of fibre added to food products may help prevent bowel disease, ischemic heart disease, and diabetes [34].

In terms of carbohydrates, no significant difference ($p>0.05$) was observed between the samples, although the carbohydrate content was higher in the control sample than in the sample containing RSF. The carbohydrate content may be attributed to potato and the breadcrumb used as a binder for the patty, as these ingredients contain a high amount of carbohydrate. The result is also consistent with the study conducted by Noroul-Asyikeen et al. [11], in which the carbohydrate content of white bread decreases with an increase in the percentage of RSF.

Table 3. Comparison of proximate composition of plant-based patties with RSF and without RSF.

	Samples	
Composition	Control	RSF15
Moisture (%)	78.66 ± 1.58^a	72.13 ± 0.61^b
Ash (%)	1.38 ± 0.28^a	1.53 ± 0.25^a
Protein (%)	2.78 ± 0.67^b	4.54 ± 0.44^a
Fat (%)	0.08 ± 0.01^b	0.14 ± 0.02^a
Fiber (%)	1.01 ± 0.28^b	2.11 ± 0.51^a
Carbohydrate (%)	16.90 ± 2.14^a	15.70 ± 1.23^a

Control: plant-based patty without addition of rubber seed flour; RSF15: plant-based patty with 15% addition of rubber seed flour. Data represents mean values ± standard deviation from 3 replicates. ^{ab}Means ± standard deviation with different letters within the same row are significantly different at $p < 0.05$.

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Physical properties of plant-based patties

Texture profile analysis

The textural characteristics of the cooked plant-based patty are shown in **Table 4** for hardness, cohesiveness, springiness, and chewiness. Based on the results, the textural attributes of hardness, cohesiveness, and chewiness showed an increasing trend, while springiness showed a decreasing trend with the addition of rubber seed flour. RSF15 showed a higher hardness value, with a significant difference ($p<0.05$) compared to the control. The lower hardness of the control sample may be related to the higher moisture content of oyster mushrooms [14]. According to a previous study, the hardness of the plant-based patty decreased in direct proportion to the amount of oyster mushroom. Moreover, the amount of oyster mushrooms added also lowered the consistency of plant-based patties correspondingly [14]. In addition, the increase in hardness with the addition of RSF was similarly observed in the study conducted by Noroul-Asyikeen *et al.* [11] for a bread product, where an increase in hardness was observed with an increase in rubber seed flour percentage.

As for cohesiveness, RSF15 showed the highest cohesiveness (0.53%), which was significantly higher than the control (0.38%). According to Sharima-Abdullah *et al.* (2018), cohesiveness was defined as the tendency and intermolecular forces within the sample to keep the components together. The addition of the rubber seed to the patty has been approved, as it has the strongest interaction with other ingredients. However, substituting oyster mushrooms with 15% RSF (RSF15) reduces springiness. The springiness of the plant-based patties is affected by the amount of oyster mushrooms, as it contributes to their meat-like texture. A previous study by Moorthi et al. [15] reported that increasing the ratio of oyster mushroom to chickpea increases springiness. Moreover, RSF15 showed a higher value for chewiness (0.14%) compared to the control (0.08%), although no significant difference ($p>0.05$) was observed between the samples. According to a previous study by Lopez-Vargas *et al.* [35] on burgers formulated with albedo fibre powder, an increased amount of fibre affected the texture of chewiness. This is similar to the current study, which observed a higher fibre value for the RSF15 sample compared to the control sample (**Table 3**).

Table 4. Textural properties of cooked plant-based patty.

	Control	RSF15
Hardness (N)	11.20 ± 1.69^b	16.20 ± 0.61^a
Cohesiveness	0.38 ± 0.08^b	0.53 ± 0.05^a
Springiness	0.55 ± 0.70^a	0.41 ± 0.52^b
Chewiness (N)	0.08 ± 0.01^b	0.14 ± 0.02^a

Data are presented as the mean ± standard deviation with N=3.
^{ab} Means with the different letters in the same row are significantly different ($p<0.05$).

Colour analysis

The colour profile of plant-based is shown in **Fig. 2**. According to the results, the L^* value for uncooked patties ranged from 61.73 to 59.83, and a significant reduction ($p<0.05$) in lightness was observed for both control and RSF15 patties after cooking, with values of 54.62 to 55.08. Comparing the control sample and RSF15, no significant difference ($p>0.05$) was observed in the cooked samples, whereas the uncooked samples showed a significant difference in lightness ($p<0.05$).

The significantly higher value of lightness observed for the control sample as compared to RSF15 for uncooked patties was attributed to the higher percentage of oyster mushroom, which provides a greyish-white natural colour to the patties, whereas the addition of RSF resulted in darker-coloured patties. Similar results were previously observed in RSF-supplemented bread, where the addition of RSF reduced lightness [11]. The a^* value indicates the redness of the plant-based patty, with higher values observed for samples containing rubber seed flour. The uncooked patty showed a significant difference ($p<0.05$) between the control and RSF15 samples, whereas no significant difference ($p>0.05$) was observed between the cooked samples. The a^* value for uncooked patties was in the range 0.23 to 2.30, while the value ranged from 2.86 to 3.88 for cooked patties.

The higher redness (a*) value for cooked patties is due to the development of brown colour during frying. Furthermore, the significant increase ($p<0.05$) in redness for uncooked patties with the addition of RSF is attributed to the brown coloured RSF. According to Abu Hasan *et al*. [25], higher a* value was observed in muffins due to the addition of rubber seeds, as the drying process of rubber seeds leads to the development of brown colour. The yellowness (b^*) for uncooked and cooked samples showed no significant difference ($p>0.05$) between the control and RSF15 samples. The b^* value for uncooked patties ranged from 12.92 to 13.67, while for cooked patties, it ranged from 14.09 to 15.56. Adding rubber seed to the patty reduces its yellowness. The yellowness of the patties may be attributed to the presence of breadcrumbs and potatoes. A similar study was conducted by Abu Hasan et al. [25], in which the yellowness value decreased when rubber seed flour was added to the muffin formulation.

Cooking properties

The cooking properties of the plant-based patty are shown in **Table 5**. Based on the results, a significantly higher percentage reduction in diameter and cooking loss was observed in the control samples compared to RSF15. The higher diameter and cooking loss could be attributed to the extensive loss of moisture during cooking [36]. Frying resulted in water evaporating, leading to weight loss of the product. Thus, the greater reductions in diameter and cooking loss for the control samples may be attributed to the higher moisture content of the uncooked patties, which was evaporated during cooking.

The higher moisture content is due to the higher percentage of oyster mushroom, as similar results were previously reported by Moorthi et al. [15], who found that an increase in oyster mushroom led to higher cooking loss for meatless nuggets. Moreover, the higher cohesiveness of the RSF15 patties may have contributed to lower cooking loss, as the tendency for the components to be held together was higher.

Table 5. Cooking properties of plant-based patties with and without RSF.

	Control	RSF15
Reduction in diameter (%)	15.15 ± 0.51^a	10.50 ± 0.57^b
Cooking loss (%)	7.67 ± 0.05^a	6.02 ± 0.05^b

^{ab} Means ± standard deviation was taken from six replications (N=6) with different letters within the same row indicating a significant difference at $p<0.05$.

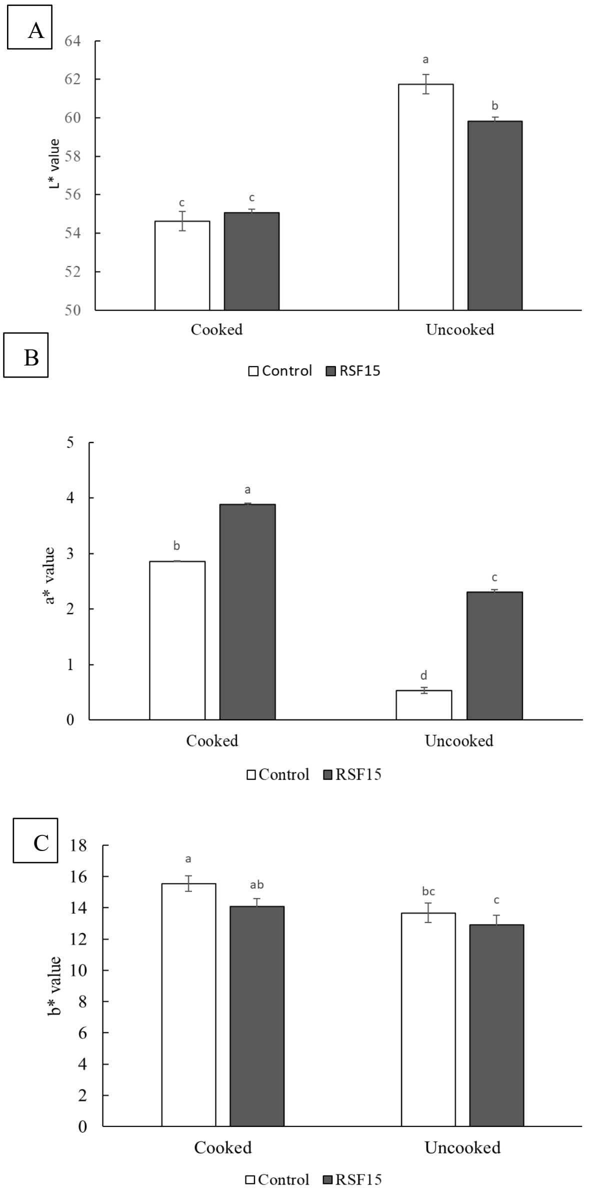

Fig. 2. Colour parameters of cooked or uncooked plant-based patties produced with and without RSF with *L**=lightness (A), *a**=redness (B) and *b**=yellowness (C). [abc] Mean values with different letters indicate significant differences at $p<0.05$. Bars represent standard deviation taken from six replicates (N=6).

Water holding capacity (WHC)

The water-holding capacity of plant-based patties formulated with different levels of oyster mushroom and rubber seed flour is shown in **Fig. 3**. Based on the observation, the RSF15 sample showed a significantly higher percentage of WHC ($p<0.05$) compared to the control, with 85.63% and 80.56%, respectively. The results showed that the addition of rubber seed flour improved the water-holding capacity of the meatless patties. According to Udo et al. [12], the highest fiber and protein levels can hold more water via adsorption, which may help prevent structural breakdown. In addition, the increase in water-holding capacity helps prevent cooking loss, as observed in the current study (**Table 4**). This is due to the significantly ($p<0.05$) higher fiber and protein content of the RSF15 patties as compared to the control. This finding is also confirmed by Noroul-Asyikeen *et al.* [11, who found that composite flour containing RSF has higher protein and fiber content, which bind more water than oyster mushrooms.

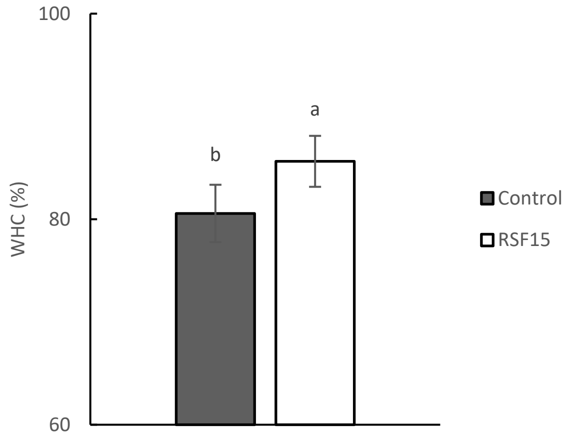

Fig. 3. Water holding capacity (WHC) of plant-based patties. Bars represent the standard deviation taken from 3 replicates. Mean values with the same letter indicate no significant difference at $p>0.05$.

CONCLUSION

This study revealed the potential of RSF for producing plant-based patties. The addition of RSF leads to an increase in ash, protein, crude fiber, and crude fat content. A slight change in colour was also observed in patties containing RSF, resulting in darker patties. Moreover, RSF also significantly affects the texture of the patties, improving water-holding capacity and reducing cooking loss. In conclusion, RSF can be used as an alternative ingredient to soy to produce plant-based patties and may be used to produce other meat analogue foods, which are usually soy-based. A future study should be conducted to improve the sensorial properties of the patties, especially taste, and to determine their potential as an ingredient in the development of various food products.

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