

*Original Research Article*

## Textural Characteristics of Oyster Mushroom–Soy Protein Extrudates and Master Curve of Specific Mechanical Energy

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**Abstract:** The effects of screw speed and oyster mushroom (OM) addition on the texture characteristics (hardness, gumminess, and chewiness) and specific mechanical energy (SME) were determined via factorial experiment design. Increasing screw speeds alone exhibited an insignificant effect ( $p > 0.05$ ) compared to the effect exhibited by OM addition. The combined effects of screw speed and OM addition significantly reduced ( $p \leq 0.05$ ) all the texture characteristics. The hardness (3521.35 g) and gumminess (2717.85) of the meat analogue extruded at the maximum screw speed of 160 rpm and 15% OM addition were close to the characteristics of the chicken breast, respectively. The OM–soy protein (SP) extrudates were chewier than the non-hydrated texturised vegetable protein (TVP) and chicken meat. The SME values of the single-screw extrusion were linearly affected by the increasing screw speed, which also lies in the range  $< 200$  kJ/kg for producing meat analogues with acceptable characteristics. Applying the superposition technique has successfully shifted the individual curves of the SME into a smooth master curve horizontally, allowing an interpolating in the prediction of SME value at a given screw speed value. An adequate low-grade OM content and extrusion screw speed value in the SP-based meat imitation provide hardness and gumminess that resembles the chicken breast. A prediction value on the specific mechanical energy of the SP-based meat analogue extrusion may be interpolated by a given screw speed value by using an established master curve.

**Keywords:** oyster mushroom; screw speed; specific mechanical energy; textural characteristics; master curve

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## 1. Introduction

The farming and meat industries in the world have been facing enormous challenges, such as arable land shrinkage, intensification of agricultural production, and livestock challenges to meet the world's meat demand (Gerhardt *et al.*, 2020). These factors lead to the importance of plant-based meat in the current trend of a healthy diet and as a future sustainable food. The Food and Agriculture Organization (FAO) has reported that the intake of meat analogues by consumers in developing countries will continue to increase by up to 73% by 2050 (FAO, 2020). In addition, plant-based meat is highly marketed in Western countries, and Asian countries will also become a future market due to growing interest in meat imitations (Ismail *et al.*, 2020).

Traditionally, meat analogues have been produced through simple processing or fermentation processes that failed to exhibit the mimic texture of animal meat. However, with the modern advanced technology in food processing and food science, meat analogue products mimic aesthetic qualities (e.g., texture, taste, and look), and the functionality of animal meat products can be produced (King & Lawrence, 2019). The extrusion cooking technique is commercially applied in the production of meat analogues. However, determining the correct extruder parameters and ingredient formulation to enhance the appearance, taste, and texture of meat analogue is the most challenging feature when it comes to extrusion cooking. Specifically, determining the right extrusion temperature is important to ensure the completion of the denaturation of proteins during extrusion. Meanwhile, screw speed is crucial to provide the shearing effect in the process of realignment and formation of the fibrous structure of the meat analogues.

On the other hand, adding extra element of health-promoting ingredients is an approach to make meat analogues more nutritious and improve their texture. Oyster mushrooms (OM) (*Pleurotus sajor-caju*) are widely planted and consumed in Malaysia because of their simple planting cycle and great taste (Mat Amin *et al.*, 2013). OM is low in fat and calories but is rich in protein, dietary fibre, vitamins, and minerals (Wan Rosli *et al.*, 2015). Additionally, all parts of the mushrooms are edible. However, a low-grade OM has unwanted parts, categorised as plantation waste. According to the Federal Agricultural Marketing Authority (FAMA), OM with a small-cap size ranging from 3 to 5 cm and a deformed shape belongs to the low-grade category (FAMA, 2017). Thus, when compared with high-grade mushrooms, they are usually sold at a much lower price or are rejected as waste.

Many extrusion processes have been using a single-screw extruder for plant protein-based meat analogue production (Omohimi *et al.*, 2014; Parmer Jr *et al.*, 2004; Rehrah *et al.*, 2009; Thadavathi *et al.*, 2019). However, there is yet any study conducted to find the relationship between varied screw speeds and the addition of OM on the texture of soy protein (SP)-based meat analogue and the extruder's specific mechanical energy (SME). Thus, the combined effects of extrusion screw speed and low-grade OM addition on the

texture characteristics (hardness, gumminess, and chewiness) of in forming an OM–SP meat analogue were analysed. The obtained texture characteristics were compared with commercial products such as hydrated non-extruded texturised vegetable protein (TVP) and chicken meat. The relationship between SME and screw speed was determined from a master curve developed using the superposition technique. A master curve is developed to display a single smooth line to express the extruder's performance at a wide range of screw speeds applied for this type of meat analogue extrusion.

## 2. Materials and Methods

### 2.1. Materials

Low-grade grey OM was obtained from Ladang Tanaman Cendawan, Universiti Putra Malaysia, Serdang, Malaysia. SP concentrate and isolated soy protein (ISP) were purchased from Shaanxi Jintai Biological Engineering Co., Ltd., China, and Imaherb Biotech Co., Ltd., China, respectively. Commercial wheat flour (Syarikat Faiza Sdn. Bhd.), regular single refined rock salt (Halagel (M) Sdn. Bhd.), and cooking palm oil (FFM Berhad) were purchased from a local shop in Serdang, Malaysia. Additives such as food-grade soy lecithin and sodium metabisulfite were acquired from Evergreen and Engineering Resources, Malaysia. Distilled water was obtained from the Packaging and Preservation Laboratory, Universiti Putra Malaysia, Serdang, Malaysia. Reference samples namely, chicken meat (drumstick and breast) and non-extruded TVP, were purchased from a local shop in Serdang, Malaysia.

### 2.2. Processing of Grey Oyster Mushroom

Fresh low-grade OM were washed to remove dirt and unwanted particles, and left in a strainer for 15 min to remove excess water. They were then mashed using an electrical chopper (HR1393/01, Philip, Malaysia) for 1 min. Based on the analyses performed according to the Official Methods of AOAC 16th Edition (AOAC, 1995), a low-grade OM contains 91.6% moisture, 3.3% protein, < 0.1% fat, 4.5% carbohydrates, 0.6% ash, and 3% dietary fibre.

### 2.3. Preparation of Feed Mixtures

A SP mixture (feed mixture) was prepared by mixing 50% SP concentrate and 50% ISP. Other ingredients, such as wheat flour, soy lecithin, sodium metabisulfite, salt, cooking palm oil, distilled water, and OM, were added to the SP mixture on a weight percentage basis. The feed mixture without OM addition, 0%, acts as a control. Other additions of OM in the mix were 7.5%, and 15%, respectively. Based on our recent work (Mazlan *et al.*, 2020), a narrow interval OM% value exhibited no distinction output. Furthermore, a feed formulation containing 15% OM became a limit to produce smooth-surfaced extrudates. Beyond 15% OM addition in the mixture produced unsatisfactory criteria extrudates. Thus, only 0%, 7.5%, and 15% were suitable to vary in the present study. Table 1 presents the detailed ingredient

formulations of the feed mixtures on a weight basis (wb%). These feed mixtures were mixed using a kitchen hand mixer (SMX-2758, Sinbo, Turkey) and kept in airtight containers. The feed mixtures were refrigerated overnight before extrusion to ensure well hydration. After hydration, the feed mixture moisture content achieved 30%–45%. An electronic moisture analyser (MX-50 Moisture Analyser, Mettler Toledo, Greifensee, Switzerland) was used to determine the moisture content.

**Table 1.** Ingredient formulations at different percentages of oyster mushroom content.

Mixture no. (wb%)	Soy proteins		Additional ingredients (% by total weight of the soy proteins)						
	SP	ISP	Wheat	Soy	Sodium	Salt	Cooking	Distilled	OM
			flour	lecithin	metabisulfite		palm oil	water	
1	50	50	2	0.4	0.18	3	10	70	0
2	50	50	2	0.4	0.18	3	10	70	7.5
3	50	50	2	0.4	0.18	3	10	70	15

#### 2.4. Extrusion Cooking

Extrusion cooking was performed using a laboratory-scale single-screw Brabender 19/20D extruder (Brabender GmbH and Co., Duisburg, Germany). The extruder has a grooved barrel with a length/diameter ratio of 20:1 and comprises of two heating zones, with an additional die heating element. A flat sheet die head 20 mm wide and 2 mm high was attached to the extruder's end without the air cooling system. Extruder temperatures were set at 80 °C (feeding zone), barrel temperature (140 °C) (compression zone), and 45 °C (die) for the first zone, second zone, and at die, respectively. A compression screw (compression ratio 2:1) was used to extrude the meat analogue. Feed mixtures were fed through a standard bin feed hopper with a dosing screw rotating at 25 rpm. The extrusion screw speed was set at 110–160 rpm. The extrudates were collected once the extrusion condition reached a steady state, as indicated by the consistent values of extruder motor torque and die temperature (zone 3) values. Samples were allowed to cool down at room temperature before being packed in polyethylene zip lock bags. Finally, the extrudates were stored in a freezer at −18 °C until further analysis.

#### 2.5. Texture Profile Analysis Measurement Technique

Texture profile analysis (TPA) was used to determine the sample's hardness, gumminess, and chewiness using a TA-XT<sub>plus</sub> Texture Analyser (Stable Micro Systems, Surrey, UK). Test samples were OM– SP extrudates produced in the laboratory, chicken meat (breast and drumstick), and non-extruded TVP. Chicken meat and non-extruded TVP were used as reference samples to compare the texture characteristics. Cooked samples were prepared by boiling water for 30 min and left to cool at a temperature of 28 ± 1 °C for 30 min. All samples were compressed twice using a 20-mm cylindrical probe (P/20) to 50% of

their original height at a constant speed of  $0.2 \text{ mms}^{-1}$  (test speed). The time elapsed between compressions was 1 s, and the post-test and pre-test speeds were set at  $0.5 \text{ mms}^{-1}$ . The hardness, gumminess, and chewiness of the extrudates were determined from a force–time (gram–second) plot obtained. These characteristics were reported in the mean of two measurement values.

### 2.6. Calculation of Specific Mechanical Energy

The SME is defined as the extruder's total energy to extrude 1 g of meat substitute in the unit of kJ/kg. SME is determined according to Equation. (1) (Godavarti & Karwe, 1997). The mean of duplicate SMEs was reported.

$$\text{SME, (kJ/kg)} = \frac{2\pi \times n \times T}{\dot{m}}, \quad (1)$$

where

$n$  = screw speed (rpm)

$T$  = motor torque at steady-state condition (Nm)

$\dot{m}$  = mass flow rate (g/min)

The laboratory-scale single-screw Brabender 19/20D extruder (Brabender GmbH and Co., Duisburg, Germany) was equipped with a torque gauge to measure the extrusion screw rotating force against the barrel wall. Torque,  $T$  value, was directly recorded by the software Brabender® measuring program (Version 4.4.0. from Brabender® GmbH & Co. KG, Duisburg, Germany) in the unit of Nm. Torque value at steady state condition was determined from a constant torque versus time curve trend during extrusion. The torque at steady state recorded is the mean of three data.

The mass flow rate of extrusion,  $\dot{m}$ , (unit in kg/s) was determined by measuring the mass of the extrudate passing the extruder in 1 min. Extrudates were collected during steady-state extrusion flow, as indicated by the constant extrusion torque mixing curve. The mass of extrudates was weighed using an analytical balance (Mettler Toledo, Greifensee, Switzerland). Each measurement was taken in duplicate, and the data were averaged.

### 2.7. Master Curve of SME

Average SME value curves were found for each OM addition level (0%, 7.5%, and 15%); thus, three curves were drawn. Then, using the superposition technique, these three curves were converted into one master curve. This procedure is further explained in the following paragraphs.

First, each of the OM addition's SME curves was plotted in a normal scale graph versus the extrusion screw speed. Next, the curve fitting for SME values was determined using the solver function in Microsoft Excel 2016 (Vista Edition, Microsoft Corporation, Albuquerque, NM, USA). Next, the SME curves were fitted to polynomial functions to

determine the relationship between increasing screw speed and the SME. The coefficient of determination  $R^2$  was determined for each OM addition to choosing the best-fit line plot (Equation [2]). The root mean square error (RMSE) was used to estimate the standard deviation of the experimental value from the model's prediction (Equation [3]). The high  $R^2$  and low RMSE values determined the best-fit line plot.

$$R^2 = 1 - \frac{SSE}{SST} = \frac{\sum (Y_{\text{experimental}} - Y_{\text{model}})^2}{\sum (Y_{\text{experimental}} - \bar{Y}_{\text{experimental}})^2} \quad (2)$$

where

SSE = sum of square error

SST = total sum of squares

$Y_{\text{experimental}}$  = experimental value of the dependent variable

$Y_{\text{model}}$  = model value of the dependent variable

$\bar{Y}_{\text{experimental}}$  = mean of the experimental value of the dependent variable

$$\text{RMSE} = \sqrt{\left[ \sum_{i=1}^N (Y_{\text{experimental},i} - Y_{\text{model},i})^2 \right] / N} \quad (3)$$

where

$N$  = the number of data

In the second step, the dimensionless shift factor,  $\alpha$ , was calculated from the selected polynomial equation to make a horizontal shift of other OM additions (7.5% and 15%) at the screw speed axis to obtain a smooth curve. Again, the highest OM addition of 15% was chosen as the reference line.

## 2.8. Statistical Analysis Technique

The mean and standard deviation values were calculated using Microsoft Excel 2016 (Vista Edition, Microsoft Corporation, Albuquerque, NM, USA). The error bars in the graphs are the standard deviation of mean values. A two-factor general full factorial design was performed to investigate the effect of screw speed and OM addition on the extrudate's texture parameters and SME using Minitab 17 software (Minitab Inc., State College, PA, USA). Data were analysed using analysis of variance (ANOVA), and the significance level was determined at  $p \leq 0.05$ . The linear Pearson coefficients ( $r$ ) correlation between the specific mechanical energy and the textural characteristics was also assessed.

### 3. Results and Discussions

#### 3.1. The Effect of Screw Speed and Oyster Mushroom Addition on Textural Characteristics

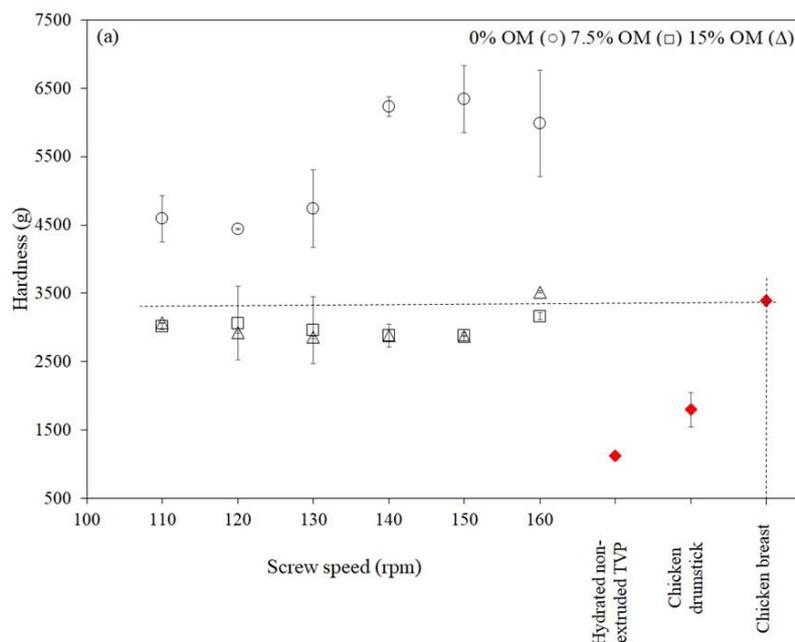
Texture characteristics obtained from the TPA of meat analogue are an important food quality feature that represents the mouth's biting sensory of the meat analogue. The textural characteristics were determined from a force–time curve of a double compression applied to the extrudates. Figure 1 shows the textural characteristics (hardness (a), gumminess (b), and chewiness (c)) of OM–SP meat analogue extruded at different screw speeds and OM addition percentages. Overall, each extrudate's gumminess and chewiness (0%, 7.5%, and 15% OM) followed the same trend as the hardness attribute because they were all hardness related, according to the definition of gumminess and chewiness by Stewart *et al.* (2012). A correlational analysis further supported the current finding, which revealed a positive correlation ( $p \leq 0.05$ ; Table 2) between chewiness and gumminess and the hardness of extrudates. Comparable data of hardness (maximum compression force) were reported by Rehrach *et al.*, (2009) where it was found that the hardness of texturised peanut protein was determined in the range of 2000–3000 g. The extrudates with the addition of OM exhibited hardness values in this range. All TPA characteristics were the highest for the control extrudates (0% OM), whereas the lower values were displayed for the extrudates with oyster mushrooms. The hardness, gumminess, and chewiness of extrudates were reduced significantly ( $p \leq 0.05$ ; Table 2) by the addition of OM up to 15%

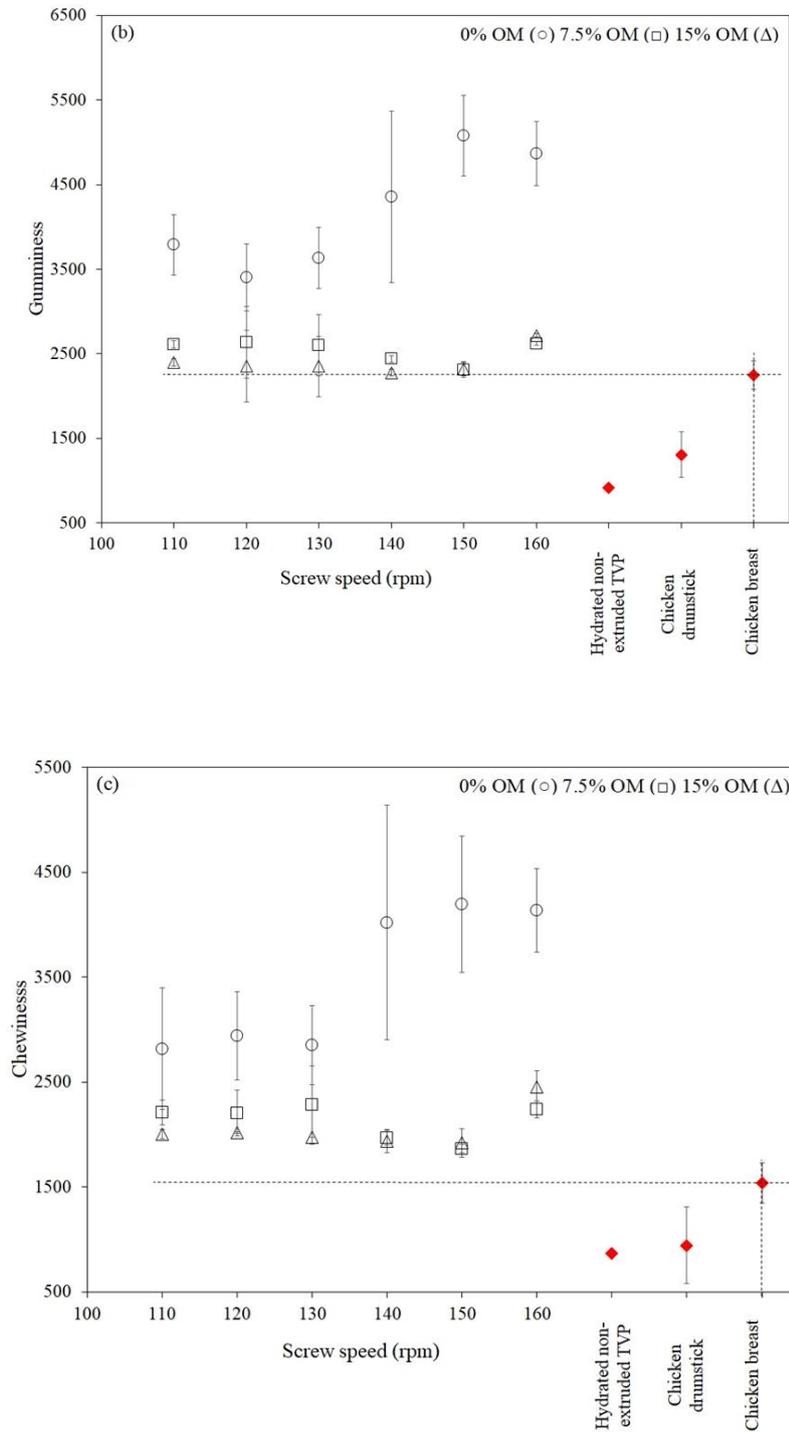
Increased moisture content in OM–SP extrudates after incorporating OM could affect the textural changes. This reason is consistent with the reported results in a recent study where the OM–SP extrudate's moisture content increased with the addition of OM (Mazlan *et al.*, 2020). Although an increase in the additional ingredient containing fibre is always associated with increased textural characteristics, increasing moisture content with the addition of OM may overcome the effect of the presence of fibre. A study that investigated the texture characteristics of chicken patties incorporated with grey OMs reported that the hardness, gumminess, and chewiness decreased proportionally with the level of OM (Wan Rosli *et al.*, 2011). The decrease could be attributed to the higher moisture retention in samples with nonmeat ingredients such as OM added to high-protein food (Yahya & Ting, 2020).

The effect of screw speed significantly affected the texture characteristics of the OM–SP extrudate ( $p \leq 0.05$ ; Table 2). The magnitude of the changes elicited by the screw speed characteristic was small compared to the changes elicited by the OM addition. However, the interaction between the screw speed and OM addition significantly affected the characteristics ( $p \leq 0.05$ ; Table 2). The extrudate's texture characteristics with no OM increased at the screw speed of 110–150 rpm and then decreased at the speed of 160 rpm. Similarly, the data in a study conducted by Mazlan and co-workers (2020) showed that the control extrudate puffed and had the highest expansion ratio at the screw speed of 160 rpm. As shown in Figure 1, the texture characteristics of extrudates containing 7.5% and 15% OM

decreased at the screw speed of 100–150 rpm and then increased at the speed of 160 rpm. Parmer Jr and co-workers (2004) reported the challenges of obtaining texturized SP meat analogue via a single-screw extrusion. Typically, a single-screw extruder has a shorter and narrower screw than a twin-screw extruder, resulting in limited energy input within the barrel. However, the present study has shown that the combined effects of screw speed and OM addition improved the texture characteristics (hardness, gumminess, and chewiness) of the extrudates at a screw speed of 160 rpm. This finding aligns with the high texturisation index and defined fibrous structure in the SP extrudates added with 15% OM (Mazlan *et al.*, 2020). These product attributes were produced at the highest screw speed of 160 rpm via single-screw extrusion. Additionally, a significant negative relationship ( $p \leq 0.05$ ; Table 2) between each textural characteristic and specific mechanical energy was exhibited. Extrudates with low values for hardness, gumminess, and chewiness are thought to be created by extrusion at higher screw speeds (160 rpm), which reflected a greater specific mechanical energy.

In the double compression test, the hydrated non-extruded TVP and chicken drumstick showed a lower hardness, gumminess, and chewiness than all the OM–SP extrudates. However, the hardness and gumminess of extrudates added with 7.5% and 15% OM were near the chicken breasts except for chewiness. Such results suggest that extrudates containing OM showed a considerable resemblance to the chicken breast texture (in terms of hardness and gumminess) than the control extrudates (without OM content).





**Figure 1.** Hardness (a), gumminess (b), and chewiness (c) of OM–SP extrudates at different screw speeds (rpm) and at different percentages of OM addition (0% (○), 7.5% (□), and 15% (Δ)). Cooked chicken breast, chicken drumstick, and hydrated non-extruded TVP (◆) were tested for comparison.

*Note:* Data were reported in the mean of two measurement values. The error bars in the graphs are the standard deviation of mean values.

**Table 2.** Coefficients of general factorial regression and correlation coefficients for the SME and textural characteristics of the extrudates

<b>Coefficients of general factorial regression</b>					
		<b>Hardness (g)</b>	<b>Gumminess</b>	<b>Chewiness</b>	<b>SME</b>
<i>p</i> -value	OM	0.0001****	0.0001****	0.0001****	0.0001****
	SS	0.003**	0.038*	0.103	0.0001****
	OM*SS	0.002**	0.015*	0.049*	0.0001****
R <sup>2</sup>		0.97	0.94	0.89	0.99

<b>Pairwise Pearson Correlations Coefficients</b>					
		<b>Hardness (g)</b>	<b>Gumminess</b>	<b>Chewiness</b>	<b>SME</b>
<b>Hardness (g)</b>		1			
<b>Gumminess</b>		0.959****	1		
<b>Chewiness</b>		0.943****	0.978****	1	
<b>SME</b>		-0.565****	-0.533***	-0.488**	1

\*, \*\*, \*\*\*, \*\*\*\* = significant at  $p \leq 0.05$ ,  $p \leq 0.01$ ,  $p \leq 0.001$ ,  $p \leq 0.0001$ , respectively

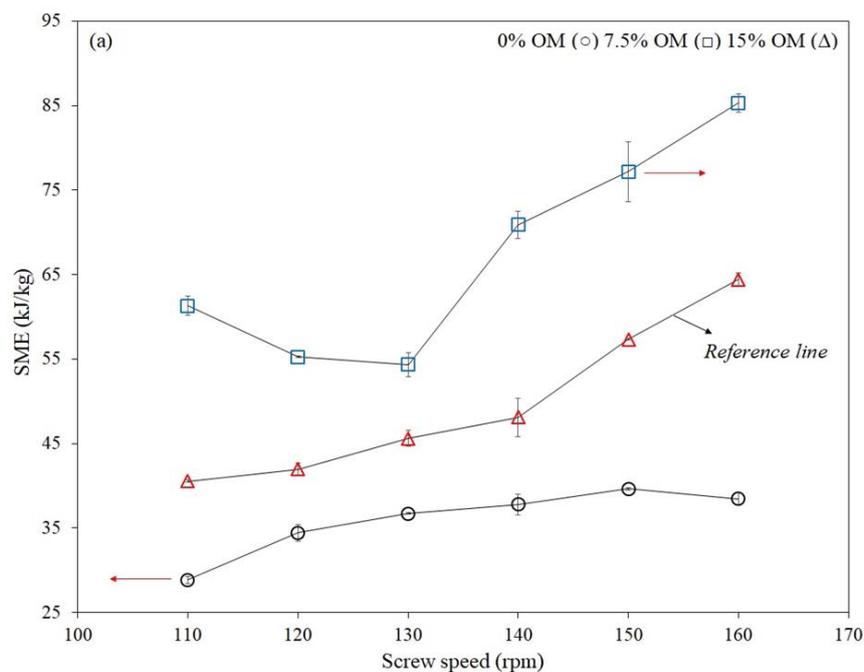
Note: OM, oyster mushroom addition (%); SS, screw speed (rpm); SME, specific mechanical energy (kJ/kg)

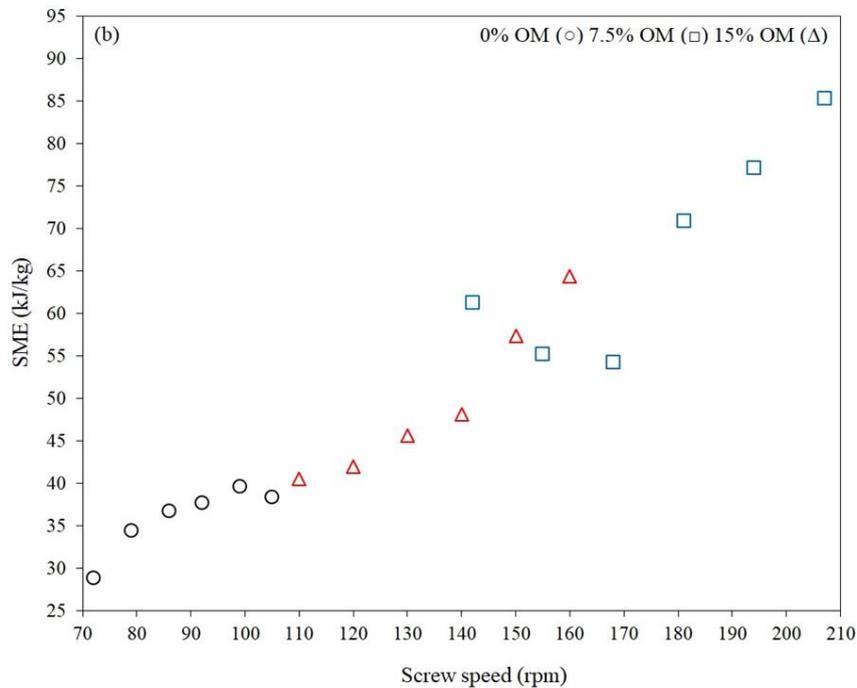
### 3.2. Master Curve of SME

Figure 2 presents the effect of screw speed on SME and its respective master curve under different percentages of OM addition. The SME values in the range of 200 kJ/kg or lower are required to form the fibrous structure in meat analogue (Bouvier & Campanella, 2014), and the efficiency of SME for the extrusion process must be lower than 1000 kJ/kg (Camacho-Hernández *et al.*, 2014). As shown in Figure 2(a), SMEs ranging from 28–85 kJ/kg were obtained from the extrusion at different screw speeds and percentages of OM addition. However, the SME values were lower than those reported for the extrusion of meat analogues using a twin-screw extruder. On the other hand, Caporgno and co-workers (2020) obtained a higher range of SME (60–160 kJ/kg) from the extrusion of SP added with microalgae using a twin-screw extruder, probably due to higher energy input from the shear force and heat transfer during extrusion.

Table 2 indicates that the interaction between screw speed and OM addition significantly affected ( $p \leq 0.05$ ) the SME. When the screw speed increased, the SME increased. A higher SME resulted in extrudates with higher hardness, gumminess, and chewiness, as previously discussed. This result agrees with Fang *et al.*, (2014) who studied the effect of SME on texturised SP hardness. However, with the addition of OM up to 15%, lower SME values were observed at increasing screw speeds. It is possible due to the increase in the protein mixture's moisture content when OM addition increases. When moisture content increased, the dough's viscosity reduced, resulting in decreased friction between the dough, extrusion screw, and barrel wall. Therefore, the force required to push the dough through the die decreased, thus also reducing the shear rate and extrusion torque (Kantrong *et al.*, 2018; Lin *et al.*, 2000).

Figure 2(b) is a master curve that displays the extrusion SME constructed from different OM addition measurement curves. The master curve describes how SMEs respond over a large range of OM addition (0–15 %) at a reference OM percentage of 15 %. From Figure 2(a), the SME increases dependence on the increasing screw speed. The individual curves of SMEs have the same shape. Each curve fits a linear model. Table 3 shows that all three linear curves have high  $R^2$  values ranging from 0.779–0.92 and RMSE = 2.0499–7.0616. Based on the SME equation (Equation 1), the SME is proportional to screw speed; thus, this linear trend was expected ( $n$ ). The data were also fitted to different polynomial equations (e.g., quadratic and cubic). However, the linear model established the best relationship between the SME and screw speeds. Thus, this similarity allows for smooth horizontal shifting along the screw speed axis. The curves measured at OM addition lower than the reference OM percentage were shifted to a higher OM percentage so that the individual curves of the SME lay in a single master curve. The red arrows in Figure 2(a) denote the shifted directions. The curve with 0% OM addition was horizontally shifted to the left, whereas the curve with 7.5% OM addition was horizontally shifted to the right. With the combined effect of the extruder's screw speed setting and ingredient formulation changes (0–15 % OM addition), it was possible to construct a master curve based on the superposition principle that showed the entire SME performance for single-screw extrusion. This information provides a proper understanding of the resulting master curve, allowing one to analyse the energy-related dynamics and verifies if the dynamic process parameter is suitable for this product manufacturing application.





**Figure 2.** Effect of screw speed on SME at 0% (○), 7.5% (□), and 15% (△) of oyster mushroom addition (a) and its respective master curve (b).

**Table 3.** Coefficients of regression fitted line plot for the SME.

Oyster mushroom addition (%)	SME (kJ/kg)		
	Fitted regression model	$R^2$	RMSE
0	Linear	0.779	2.0499
7.5		0.746	7.0616
15		0.921	2.9472

#### 4. Conclusions

This study suggested that low-grade OM mixed with SP have the potential to produce meat analogues with acceptable hardness, gumminess, and chewiness. The variation of OM addition in the mixture significantly affected ( $p \leq 0.05$ ) the extrudate's texture characteristics and the extruder's SME. A high level of OM addition (15%) reduced the texture characteristics of the meat analogue. Comparison with reference samples revealed that the hardness and gumminess value of the OM–SP meat analogue lies near the chicken breast. However, the chewiness value is slightly higher than the chicken breast. Shifting SME data to a single smooth master curve is useful for predicting the overall effect of screw speed on the extruder's performance in producing OM–SP meat analogues. The statistical analysis ( $p \leq 0.05$ ) supported that the data were linearly increased by the increasing extrusion

screw speeds. For meat analogue processing and single-screw extruder efficiency, the master curve is useful as it provides an approach to simple, fast, and effective reference to SME for operation and equipment design. Future studies can be conducted on the sensory evaluation of an OM- SP meat analogue to further grasp the consumer acceptability of extruded textural features.

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