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Physicochemical and antioxidant properties of commercial shrimp paste in Besut market

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Abstract

Shrimp paste is a popular traditional fermented seafood product and is used in cooking as a food seasoning. The unique taste and aromatic compounds of shrimp paste are made from the degradation of shrimp protein by salt-tolerant microorganisms. This study aims to compare the physicochemical composition and antioxidant activity content among different brands of shrimp paste at the local market. Four different samples of shrimp paste were labelled as SP1, SP2, SP3 and SP4. Sample SP1 showed the highest protein content (35.22 %) while sample SP4 contained the lowest protein (18.34 %). Sample SP4 showed significantly the highest moisture and ash content which were 39.25 % and 57.46 % respectively. The highest titratable acidity for sample SP4 (1.63 %) was significant in with accordance of high salinity content in shrimp paste. Sample SP1 was significantly higher (p<0.05) in free fatty acid which was 31.87 % while sample SP4 has the lowest which was 19.69 %. All shrimp paste samples showed water activity in the range of 0.72 - 0.76. Furthermore, the pH value for all the commercial shrimp paste samples was in the range of 7.00 -7.32. For the colorimetric of shrimp paste, sample SP3 had a significantly higher L* value (49.08) than sample SP4 (39.33). Sample SP4 showed the highest DPPH radical scavenging activity which was 3.68 µmol TE/g sample. These findings provide nutritional value and information to the consumer and thus help consumers decide for their choices.

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1. INTRODUCTION

A fermented shrimp paste or prawn sauce is frequently used in Southern Chinese and Southeast Asian cuisines. In Southeast Asia, there are distinguished names for this fermented product such as bagoong (Philippines), shiokara (Japan), mam ruoc (Vietnam), terasi (Indonesia), ngapi (Myanmar) and belacan (Malaysia) (Daroonpunt et al., 2016). Traditionally, ground shrimp and salt are used to make shrimp paste, which is then subsequently fermented for a few weeks before being dried outside in the sun. Before being sold, it is occasionally even formed into dried blocks (Kim et al., 2014).

Shrimp paste is prepared from Acetes species, known as *geragau* in Malaysia or *rebon* in Indonesia. It is a thick paste that ranges in colour from greyish pink to greyish purple. It tastes strongly of buttery, stingy shrimp with a hint of sweetness. Shrimp tissues undergo enzymatic breakdown during the fermentation and bacterial action assists in proteolysis and flavour development. Various techniques in terms of the period of shrimp paste fermentation and different salt concentrations used to produce shrimp paste result in composition and quality differences among products (Hajeb & Jinap, 2013). Fermented shrimp paste in Southeast Asia has a moisture content of 56.1–70.9 % and a total nitrogen component of 1.51–2.41 % (Kim et al., 2014). The moisture content in Thai fermented shrimp paste was in the range 37.36-46.85 % and ash was found to be the most dominant constituent (50.76–53.38 % based on dry matter) (Prapasuwannakul & Suwannahong, 2015). A protein made up the majority in the proximate composition about 29.44-53.27 % dry weight basis (Pongsetkul et al., 2014).

Traditional fermented fish products are often salty foods with varying salt contents. According to Ruddle and Ishige (2005), fermented fish can also be divided into three categories: highly salted (20 % salt), low salted (6–8 % salt), and products with no added salt. In addition, fish fermentation enhances mineral bioavailability and gives the product favourable organoleptic qualities such as texture, taste, odour and appearance (Narzary et al., 2021). However, lowering the salt content in the fish paste will cause the putrefaction whereas the nutritional content will be reduced (Anggo et al., 2015).

Extending the fermentation period of shrimp paste can lead to the formation of additional substances that

can contribute to an increase in overall antioxidant activity. Research has indicated that amino compounds, such as

amino acids and peptides, can play a crucial role as primary antioxidants in shrimp paste (Peralta et al., 2008). Peralta et al., (2008) observe the initial DPPH radical scavenging activity considerably enhanced from 1, 90, 180, and 360 days of fermentation.

Therefore, this study was conducted to obtain data on the physicochemical composition and antioxidant properties of commercial shrimp paste in the Besut market.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

Four different brands of shrimp paste samples were randomly purchased from the local grocery store in Besut, Terengganu. The brands of the commercial shrimp paste were namely as SP1, SP2, SP3, and SP4. The samples were chosen based on ingredients from shrimp and salt were 80- 85 % (w/w) and 15-20 % (w/w). The samples were first dried in an oven at 80 °C for further analysis.

2.2. Chemical composition analysis

Moisture, ash and protein were analysed following the Association of Official Analytical Chemists' standard procedure (AOAC, 2005). For the moisture content, 3 g of samples were weighed and the weight loss of drying at 105 °C in an oven (MMT-UF110, Memmert, Germany) was determined. Samples were dried in an oven at 105 °C until constant weights of the crucible containing the samples were obtained. The percentage of moisture content was calculated by the difference between the sample's dry weight and wet weight (Ilyanie et al., 2020).

For the ash analysis, 3.0 g dried samples were first burned and turned to ash at 525 °C for 24 hours, forming a grey material (ash), and then cooled in a desiccator. The following calculation was used to calculate the percentage of ash (Ilyanie et al., 2020) (Eq.1).

Ash (%) =
$$\frac{W_1}{W_2} \times 100$$
 (Eq 1)

Where; W1 = weight of samples after ashing, W2 = weight of samples before ashing.

Protein content was determined by the Kjeldahl method with an automatic nitrogen analyzer (Kjeltec 2300 Analyzer Unit; Foss Tecator AB). The following calculation (Eq 2 and 3) represents the crude protein percentage as the total nitrogen percentage multiplied by 6.25 (the nitrogen-to-protein conversion factor for fish and shellfish products) (Ilyanie et al., 2020).

Crude protein (%) (Eq 2)
= Nitrogen in sample (%)
$$\times$$
 6.25

$$N(\%) = \frac{[A \times (T - B) \times 14.007 \times 100]}{Weight of sample (g) \times 100}$$
(Eq 3)

where T = Volume acid for sample,

B = Volume acid for blank,

A = Normality of HCl,

F = Protein factor, 6.25/5.7/6.3

2.3. Determination of pH

Two grams of each sample were weighed respectively and homogenized with 20 ml distilled water. The pH value of the shrimp solution was measured by using a pH meter (pH211 Microprocessor).

2.4. Determination of salinity

Two grams of samples were diluted with 10 ml of distilled water. Then, a few drops of the sample were placed on the main prism of Refractometer Brix Sugar, and the daylight plate was closed. The sample was evenly distributed and air bubbles were eliminated on the prism. The front end of the refractometer was aimed at the direction of light. The reading was taken where the boundary line of blue and white crossed the graduated scale.

2.5. Determination of water activity

The water activity of each sample was measured by filling the ground sample without pores by using a water activity meter (Aqua Lab Water Activity Meter, USA). The samples were placed into a prepared sample cup. The opened PAWKIT was placed onto the prepared sample cup. The measurement of a_w was recorded when the sunburst icon remained on the screen until the device beeped. The measurements of water activity of each sample were measured in duplicate.

2.6. Determination of titratable acidity

10 g of each sample was dissolved in 30 mL of distilled water and carefully mixed. In the mixture, a few drops of phenolphthalein indicator were added. To ensure complete neutralisation, it was titrated against a standard 0.1N NaOH solution until a light pink colour lasted for between 10 and 15 seconds. The titratable acidity was calculated by the following equation.

$$Acid \% (w/w) = \frac{\text{N.V. EQ}}{W(g) \times 1000}$$
(Eq 4)

Where N = normality of titrant, NaOH (mEq/mL)
 V = volume of titrant (mL)
 Eq. wt. = equivalent weight of predominant acid
 (mg/mEq)
 W = mass of sample (g)
 1,000 = factor relating mg to grams (mg/g)
 (1/10=100/1,000)

2.7. Colour

The colour analysis of the samples was conducted using a colourimeter based on the $L^*a^*b^*$ colour scale system (Model CR – 400, Konica Minolta). In this system, the L* value indicates the lightness or darkness of the colour, the* value represents the redness or greenness, and the b* value represents the yellowness or blueness of the samples.

2.8. Free fatty acid (FFA)

The method of determination of free fatty acid was referred to Oyelese et al., (2013) with a slight modification. About 15 ml diethyl ether was mixed with 15 ml ethanol. 5g of each sample (SP1, SP2, SP3, and SP4) were dissolved in the mixture neutral solvent and titrated with aqueous 0.1M sodium hydroxide (NaOH). Dissolved 0.1M NaOH was shaken constantly until the pink colour was obtained which persisted for 15 seconds (Eg 5).

$$Acid value = \frac{Volume of 0.1N alkali}{Samples weight}$$
(Eq 5)

2.9. Antioxidant activity

2.9.1. Preparation of the soluble fraction

The soluble fraction was prepared according to Faithong and Benjakul (2012). One gram of each sample was mixed with 100 ml of distilled water, and the mixture was homogenised for two minutes using a homogenizer at a speed of 10,000 rpm. The homogenate was agitated at room temperature for 30 minutes. To remove undissolved debris, the mixture was centrifuged at 3000g for 10 min at ambient temperature using a benchtop centrifuge (Tomy LC 230, Tomy, Japan). The supernatant was kept for the antioxidant activity analysis.

2.9.2. DPPH radical scavenging activity

The DPPH scavenging activity was determined according to Faithong and Benjakul (2012). Approximately 1.5 ml of the sample was added to 1.5 ml of 0.15 mM of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) in ethanol. After aggressively mixing the mixture, it was left to stand at room temperature in the dark for 30 minutes. A UV-Vis spectrophotometer (V630, Jasco, Japan) was used to detect the solution's absorbance at 517 nm. The same steps were performed to prepare the blank, except distilled water was used in place of the sample. A standard curve was prepared using Trolox in the concentration range of 10–60 μ M. The activity was expressed as μ mol Trolox equivalents (TE)/g sample.

2.10 Statistical analysis

Data were analysed using SPSS (Statistical Package for the Social Sciences) version 24.0. A significant difference was considered at the level p<0.05. Duncan's multiple range test was also used to compare the means. Using one-way ANOVA, pair comparisons were made. Each experiment was conducted in duplicate.

3. **RESULT AND DISCUSSION**

3.1. Chemical composition of commercial shrimp paste

Table 1. shows the percentage of moisture, ash and protein in different commercial shrimp pastes. The chemical composition of moisture was significantly highest (P<0.05) in sample SP4 which was 39.25%. Sample SP1 contains the lowest moisture content which was 32.54%. According to Ali et al. (2019), the variation in moisture content of fermented shrimp paste is due to variations in pre-treatment, fermentation time, and product drying. The moisture content of Thai Kapi that was prepared from small-sized shrimps (Acetes spp.) ranged from 33.95 to 52.19 % (Daroonpunt et al., 2016). According to Handayani et al. (2021), as the moisture content in the product decreases, the texture of shrimp paste becomes firmer or higher in value. The drying process significantly influences the moisture content. Furthermore, shrimp paste with a 40 % moisture content is of high quality (Helmi et al., 2022). The presence of water in food plays a crucial role as it impacts the visual appeal, texture, and flavour of the final product. Measurement of moisture content in food is very important because the high and low moisture content in a food product will determine the final quality of a product (Handayani et al., 2021).

Ash content represents the total mineral content in the shrimp paste. Fresh shrimps themselves are rich in minerals, which are important for supporting a variety of biological activities (Ajifolokun et al., 2018). According to Prapasuwannakul and Suwannahong (2015), the amount of ash content followed by high salt content proves that the SP4 sample has the highest ash and salinity as shown in Table 2. Sample SP4 had the highest ash content (57.46 %) while sample SP1 has the lowest ash content (46.26 %). A study conducted by Ilyanie et al. (2020) found that ash content was a major component in shrimp paste which was 56.15 %. The presence of a significant amount of salt in the fermentation process resulted in a higher mineral content, leading to an increased ash content in the final products.

The highest percentage of protein was obtained by sample SP1 which was 35.22 % significantly. While sample SP4 contains the lowest percentage of protein (18.34 %). A lower protein content in SP4 samples might be because of the prolonged fermentation that resulted in the breakdown of amino acids and conversion of nitrogen compounds into ammonia by both metabolic activities of microorganisms and enzymatic decomposition (Xu et al. 2008) that led to a substantial increase in peptides (Yin et al. 2005). As a result, the fermentation time had an impact on protein hydrolysis as well as decomposition. A study conducted by Ilyanie et al. (2020) stated that the protein content of the shrimp paste sample was 31.83 % which is comparable with the result obtained. According to Pongsetkul et al. (2014), shrimp paste has a significant protein content ranging from 29.44 % to 53.17 % based on dry matter. This indicates that it can be considered a good source of proteins from the shrimp which is naturally rich in protein.

Table 1: Chemical composition of commercial shrimp paste

Sample	Chemical composition (%)		
	Moisture	Ash	Protein
SP1	34.47±0.60 ^b	49.55±0.18 ^b	35.22±0.03ª
SP2	32.54±0.12°	46.26±0.30°	26.40 ± 2.18^{b}
SP3	37.92±1.13ª	$48.90{\pm}1.26^{b}$	20.29±0.13°
SP4	39.25±0.25ª	57.46±0.77ª	18.34±0.74°

Mean values \pm standard deviation from duplicate determination. Different superscripts in the same column indicate a significant difference (p<0.05)

3.2. Physical properties of shrimp paste

The titratable acidity (TA), salinity, pH and water activity (aw) of different commercial shrimp pastes as shown in Table 2. TA in shrimp paste was expressed in lactic acid. During fermentation, lactic acid bacteria metabolize sugars present in the shrimp paste, converting them into lactic acid. The accumulation of lactic acid contributes to the characteristic acidity of the shrimp paste. Therefore, the measurement of TA in shrimp paste helps quantify the lactic acid content, among other organic acids. As shown in the result, the TA of commercial shrimp paste samples were in the range of 1.03 - 1.63 %. The highest TA was for sample SP4 with 1.63 % significantly. High titratable acidity indicates high total acid content in shrimp paste. The high level of TA is mainly associated with the major organic acid such as lactic acid that is produced via fermentation and continually accumulates toward the end of fermentation (Chadong et al., 2015).

Sample SP4 showed the highest salinity which was 7.05 % and sample SP2 contained the lowest salinity which was 4.45 %. A study by Mizutani et al. (1992), stated that the salinity of shrimp paste generally was

approximately ~17.5 %. The amount and type of salt used throughout the manufacturing process determines the salinity of shrimp paste (Cho & Kim, 2010). Kim et al. (2014) reported that the salinity of Bruneian shrimp paste was measured to be 14.94 %, which was significantly higher than the salinity of Korean dried shrimp paste (12.87 %) and Korean fermented and dried Saewoojeot paste (13.46 %). Another study by Montano et al. (2001), documented that the salinity of Filipino shrimp paste Alamang was 24.4 %.

The a_w of the samples was in the range of 0.72 - 0.76 which indicates a slightly significant difference between the SP1 and SP4 samples. Sample SP3 and SP4 both contain the highest a_w which were 0.76 while SP1 had the lowest a_w which was 0.72. It was comparable to Bruneian fermented paste and Korean dried shrimp paste which has $a_w 0.728$ and 0.771 respectively (Kim et al., 2014). The optimum level of a_w for microbiological stability is 0.7. As a result, it shows that a_w of shrimp paste is low enough to permit microbial growth at room temperature (Kim et al., 2014).

Table 2: Physical properties of commercial shrimp paste

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Titratable acidity	Salinity	рН	Water activity (a _w)
1.47±0.04 ^t	5.40±0.14°	7.32±0.23ª	0.72±0.01 ^b
1.03±0.01°	$4.45{\pm}0.07^{d}$	7.12±0.03ª	$0.75{\pm}0.01^{ab}$
1.16±0.02°	$6.60{\pm}0.14^{b}$	7.00 ± 0.02^{a}	0.76±0.01 ^{ab}
1.63±0.04 ²	$7.05{\pm}0.07^{a}$	7.17±0.04 ^a	0.76±0.01ª
	acidity 1.47±0.04 ^t 1.03±0.01 ^c 1.16±0.02 ^c	acidity 1.47 ± 0.04^{t} 5.40 ± 0.14^{c} 1.03 ± 0.01^{c} 4.45 ± 0.07^{d} 1.16 ± 0.02^{c} 6.60 ± 0.14^{b}	acidity 1 1.47 ± 0.04^{t} 5.40 ± 0.14^{c} 7.32 ± 0.23^{a} 1.03 ± 0.01^{c} 4.45 ± 0.07^{d} 7.12 ± 0.03^{a} 1.16 ± 0.02^{c} 6.60 ± 0.14^{b} 7.00 ± 0.02^{a}

Mean values \pm standard deviation from duplicate determination. Different superscripts in the same column indicate a significant difference (p<0.05)

A pH value indicates the level of acidity or alkalinity in a solution. It is a measure of the concentration of hydrogen ions in the solution. A smaller pH value signifies a higher degree of acidity, while a higher pH value indicates a lower degree of acidity. In other words, the acidity of a solution increases as the pH value decreases, and vice versa (Handayani et al., 2021). Based on the result, the pH value for all the commercial shrimp paste samples was in the range of 7.00 - 7.32. It was comparable to the pH value in a study conducted by Prapasuwannakul and Suwannahong (2015) whereas the Klongkone shrimp paste had a pH in a range of 7.01 - 7.71. In a study conducted by Ilyanie et al. (2020), shrimp paste has been found to have a basic pH of 7.31. Similar pH values were observed in shrimp paste products such as Kapi as reported by Faithong et al. (2010).

Kim et al. (2014) reported that Bruneian shrimp paste and Saewoojeot (Korean shrimp paste) have a pH of 7.56 and 7.68 respectively. Sample SP1 has the highest pH which was 7.32 while sample SP3 has the lowest pH. The pH resulted in basic pH due to fermentation breakdown products or the generation of volatile base compounds like ammonia. The small proteins or peptides may undergo breakdown as the hydrolysis continues. This might encourage the synthesis of basic molecules with low molecular weight (Pongsetkul et al., 2014).

3.3. Quality analysis of shrimp paste

The free fatty acid (FFA) of the commercial shrimp paste samples is shown in Table 3. FFA in sample SP1 was significantly higher (p<0.05) which was 31.87 %. Sample SP4 has the lowest FFA which was 19.69 %. The nutritional qualities of shrimp paste such as an increase in FFAs, may be enhanced by reducing the salt content of the food (Helmi et al., 2022). A study conducted by Peralta et al. (2005) stated that FFA content in fermented shrimp paste at initial (1 day) and end fermentation (10 days) rose from 29.7 % to 47.8 % respectively. The high content of FFAs in shrimp paste can contribute to its strong aroma. During the fermentation process of shrimp paste, endogenous and microbial enzymes both cause lipolysis in meat and fish. Free fatty acids (FFA) are liberated during lipolysis in fermented foods, where they are then subjected to additional oxidation (Pongsetkul et al., 2017). These FFAs undergo oxidation and release volatile compounds that contribute to the distinct and pungent aroma of the paste. This oxidation leads to the formation of compounds such as aldehydes and ketones, which contribute to the development of flavours and aromas in fermented foods (Pongsetkul et al., 2017).

Table 3: Free fatty acid (FFA) of commercial shrimp paste

Samples	Free fatty acid (%)
SP1	31.87±1.20 ^a
SP2	20.30±0.28°
SP3	23.58±0.32 ^b
SP4	19.69±0.56°

Mean values \pm standard deviation from duplicate determination. Different superscripts in the same column indicate a significant difference (p<0.05)

3.4. Measurement of colour

Table 4. shows the colourimetric value of commercial shrimp paste. Sample SP3 showed a significantly higher L* value which was 49.08. This was shown by the lighter brown colour compared to sample SP4 which was darker brown (Figure 1). This may be due to the different pigment contents in the raw materials, the manufacturing process, and the additives added such as natural colouring agents like paprika and rose may contribute to the colour variance of the shrimp paste. Both

enzymatic and non-enzymatic processes, such as active polyphenol oxidase and the Maillard reaction, can cause shrimp paste to turn brown (Daroonpunt et al., 2016). Handayani et al. (2021) reported that high-quality shrimp paste is typically characterized by its dark brown colour, distinctive aroma of shrimp paste, absence of rancid odour, and absence of impurities.

As for the * value of the shrimp paste, it is mainly due to the presence of pigments from the shrimp itself and other ingredients used in the fermentation process. Sample SP3 had the highest a* value (5.96) compared to other samples. According to Handayani et al. (2021), the reddish brown colour of specific shrimp paste products can be affected by the natural astaxanthin pigment present in shrimp shells. The longer the fermentation process, the darker the colour of the shrimp paste produced. Additionally, it has been observed that certain shrimp products may contain added food colour additives.

The yellowness in shrimp paste is influenced by the natural pigments present in shrimp, such as carotenoids. These pigments contribute to the yellow colour of the shrimp paste. Sample SP3 also had the highest b* value (16.60) may be due to the inclusion of ingredients like turmeric or yellow spices in certain recipes can also contribute to the yellow colour.

Table 4: Colourimetric value of commercial shrimp paste

Samples	L*	a*	b*
SP1	40.44 ± 0.04^{bc}	3.73±0.11 ^{bc}	7.76 ± 0.40^{bc}
SP2	$41.08{\pm}0.08^{\mathrm{b}}$	3.06±0.01°	7.22±0.03°
SP3	$49.08{\pm}1.23^{a}$	5.96±0.71ª	16.60±0.08ª
SP4	39.33±0.08°	4.20 ± 0.07^{b}	$8.50{\pm}0.51^{\text{b}}$

Mean values \pm standard deviation from duplicate determination. Different superscripts in the same column indicate a significant difference (p<0.05)

L *, lightness; a *, redness-greenness; b *, yellowness-blueness



Figure 1: Color and appearance of (A) sample SP3 and (B) sample SP4

3.5. Antioxidant activity

Table 5. shows the DPPH assay of commercial shrimp paste. The DPPH radical scavenging activity ranged from $1.13 - 3.68 \mu$ mol TE/g sample. The Trolox

equivalent value represents the ability of a substance to neutralize free radicals and protect against oxidative stress. When determining the Trolox equivalent value, a substance is compared to Trolox in terms of its ability to scavenge free radicals or inhibit oxidative reactions. If a substance has a higher Trolox equivalent value, it indicates that it has a stronger antioxidant capacity. The different DPPH radical scavenging activity of each sample indicates the different FFA content. The highest value of antioxidant activity was from sample SP4 which was 3.68 µmol TE/g sample while sample SP1 had the lowest value of antioxidant activity which was 1.13 µmol TE/g sample. Sample SP4 had the highest antioxidant activity due to its low free fatty acid (Table 3) content whereas the high levels of antioxidants will prevent the shrimp paste from oxidative damage. Thus, sample SP4 showed a high quality in terms of antioxidants compared to sample SP1.

According to a study conducted by Faithong et al. (2010), the soluble fraction from fermented Jaloo, Koong-Som and Kapi Kapi showed DPPH radical-scavenging activity in the range of $4.01-12.10 \mu$ mol TE/g sample. Kapi showed DPPH the highest radical-scavenging activity in the range of $4.34-12.10 \mu$ mol TE/g sample. The differences in activity observed among the samples imply that the peptides or free amino acids found in the fermented products have the potential to provide hydrogen atoms to free radicals which could help inhibit the propagation process (Faithong et al., 2010).

Table 5:	DPPH	assay	of	commercial	shrimp	paste
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Sample	DPPH (µmol TE/g sample)
SP1	1.13±0.01 ^d
SP2	2.14±0.01 ^b
SP3	1.72±0.02°
SP4	3.68±0.03ª

TE: Trolox equivalents

Mean values \pm standard deviation from duplicate determination.

Different superscripts in the same column indicate a significant difference (p<0.05)

4. CONCLUSION

The study found that commercial shrimp paste had different chemical and physical composition, texture, colour and antioxidant properties. Sample SP4 shows the most chosen because has the highest moisture, ash content, TA, salinity and a_w. The result for FFAs shows that sample SP1 had the highest value. For the colour acceptance, sample SP3 sample has the highest lightness, redness and yellowness which means lighter brown in colour, reddish colour and yellowness. These findings also highlighted sample SP4 showed DPPH the highest radical-scavenging activity.

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