Sensory and nutritional properties of Chinese olive pomace based high fibre biscuit

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INTRODUCTION

In the last decades, dietary fibre has been recognised as “the seventh nutrient” due to its beneficial effects in preventing many chronic diseases (Montagne 2003). It is widely accepted that dietary fibre intake would benefit a range of health issues, such as enhancing the control of serum glucose and cholesterol levels, lowering the risk of coronary heart disease and certain forms of cancer, and improving gastrointestinal function. A number of publications also supported that shortage of fibre intake leads to chronic ailments such as diabetes mellitus, colon cancer, cardiovascular disease and obesity (Huxley et al., 2013; Post 2012; Kaczmarczyk 2012). The traditional sources of dietary fibre include cereals, beans, fruits and vegetables, however, due to the marked changes in the dietary habits, a decline in total dietary fibre intake has become a problematic trend globally. Therefore, development of fibre enriched foods has become a novel trend in food industry. Indeed, studies have been conducted to investigate the feasibility of incorporation of dietary fibre into the baked products, such as bread (Liu et al., 2016), cake (Garcia-zaragoza et al., 2010) and biscuit (Protonotariou et al., 2016) to meet the demand for low fat and high fibre food products.

Chinese Olive (Canarium album (Lour.) Raeusch.) is a kind of evergreen arbor, which belongs to burseraceae, canarium (Omer and Mohamed 2012). Nowadays, the development of olive processing industry not only witnesses the expansion of olive products (such as olive oil, olive juice, olive wine and olive vinegar), but also produces a large quantity of by-products such as olive pomace. Approximately 35–40 kg of olive pomace are released per 100 kg of olive during the olive production process (Akay et al., 2015). Disposal of these olive by-products remains a crucial problem causing the environmental pollution. Up-to-date, the olive pomace has been demonstrated to contain abundant nutritional components including dietary fibre, flavone, tannin and possessed the cardioprotective activities (Sioriki et al., 2016). Therefore, olive pomace can be explored as a good source for producing functional foods with improved nutritional properties.

ABSTRACT

Olive pomace is the major by-products of olive processing industry, which is considered as a rich source of dietary fibre and other natural bioactive compounds. The present study aimed to develop Chinese olive pomace powders incorporated biscuit and evaluate its physical, sensory and nutritional properties. The results revealed that blends of wheat flour powder (85 g), Chinese olive pomace powder (15 g), sugar powder (33 g), shortening (22 g), sodium chloride (1.1 g), sodium bicarbonate (0.7 g), ammonium bicarbonate (0.3 g), skimmed milk powder (6 g), one egg and 12.5 mL water baked at temperature 200-220 °C for 8 min yielded dietary fibre enriched biscuit with acceptable texture and appearance. The sensory evaluation showed this newly formulated biscuit received an average score of 4.5(±0.58) on a 5-point hedonic scale, suggesting the biscuit has high overall acceptability. Besides, the nutritional quality evaluation also demonstrated this olive pomace incorporated biscuit contained abundant dietary fibre with significantly lower expected glycemic index compared to the traditional wheat flour biscuits. Taken together, the results suggested that incorporation of olive pomace powder in biscuit production could be used to develop functional food with enhanced fibre abundance, high nutritional quality and acceptability, and low calorie & glycemic index.

Keywords: Biscuits; Dietary fibre; Olive pomace; Starch digestibility
Biscuits are the most popularly consumed bakery items in the world. Nowadays, incorporation of new ingredients in the traditional biscuit is a promising strategy to develop healthy and nutritious bakery products in food industry. Previous studies have demonstrated that addition of fibre rich ingredients in biscuit can improves nutritional quality of biscuit (Ashoush and Gadallah 2011b; Shiny Lizia 2014; Huang 2015). For example, high fiber biscuits containing millet (Shiny Lizia 2014) and oat bran (Khalil 2015) have been developed and become widely popular towards the consumers. Recently, incorporating mango peels and kernels powders into biscuits was also reported to enhance its nutritional quality with improved antioxidant properties (Ashoush and Gadallah 2011a).

Hence, the present work was undertaken to understand the effects of olive pomace incorporation on the sensory and nutritional properties of new formulated biscuits. The usage of olive pomace in the recipe was optimized based on the sensory evaluation and physical evaluation firstly. Furthermore, the nutritional properties of this Chinese olive pomace based biscuits were measured. Especially, the changes in the starch digestibility and expected glycemic index were investigated. In conclusion, the feasibility of incorporation of olive pomace as ingredients in biscuit production was also discussed in the present study.

**MATERIALS AND METHODS**

**Reagents**

Chinese olive pomace was received from Fuzhou Dashijie Olive Co., LTD in Fujian province, China. The olive pomace was ground to pass through 40-mesh (425 μm) sieve to obtain a homogeneous particle size of olive pomace powder. Sugar, milk powder, sodium chloride, sodium bicarbonate, and ammonium bicarbonate were purchased from COFCO, China. All enzymes were obtained from Sigma (St. Luis, USA).

**Preparation of chinese olive pomace incorporated high fibre biscuits**

To prepare the Chinese olive pomace incorporated biscuits, a traditional recipe with modification (Bartkiene et al., 2015) was optimized based on the sensory evaluation and physical evaluation. Briefly, biscuits were prepared from white wheat flour and olive pomace powder in the ratios of 100:0, 90:10, 85:15, and 80:20 respectively. White wheat flour biscuits were considered as control. The standardized formulation for the biscuits also contained following ingredients: 33 g sugar, 22 g shortening, 1.1 g sodium chloride, 0.7 g sodium bicarbonate, 0.3 g ammonium bicarbonate and 6 g skimmed milk powder, one egg (50-60 g) and 12.5 ml water per 100 g flour or flour/olive pomace mixture. The ground sugar and fat were creamed in a Hobart mixer (Hobart N50, Hobart Co., Troy, OH, USA) with a flat beater for 3 min at 61 rpm. Sodium chloride, sodium bicarbonate, and ammonium bicarbonate were dissolved in water and added. Skimmed milk powder was made into suspension with water and transferred to the cream. The contents were mixed for another 6 min at 125 rpm to obtain a homogenized and creamy texture. Sieved flour was added to the cream and mixed for 2 min at 61 rpm. The prepared dough pieces were rolled to a thickness of 4-5 mm and cut into equal sizes using a biscuit cutter and baked at temperature 220-220 °C for 8 min. The well baked biscuits were cooled to room temperature, packaged, and stored in sealed polyethylene bags till further use. Biscuits of each formulation were made three separate times.

**Sensory evaluation of biscuits**

The olive pomace-incorporated high fibre biscuit was subjected to sensory evaluation to measure the acceptance towards the sensory attributes of the biscuits using a five-point hedonic scale, where 1 = dislike extremely, 2 = dislike moderately, 3 = neither like nor dislike, 4 = like moderately and 5 = like extremely according to previous reports (Gupta, Bawa, and Abu-Ghannam 2011; Ahmed, Sayed, and Sayed 2014). The sensory evaluation was carried out by 50 panelists in the age group 20 to 50 years with normal taste sensitivity and sensory evaluation experience. The score card for the evaluation of the biscuit was provided along with instructions to each judge before evaluation. Each judge can indicate the extent of his likes or dislikes in terms of color, appearance, flavor, taste, texture/doneness and overall acceptability. Also the panelists were asked to list any defects in the samples.

**Evaluation of the nutritional properties**

To evaluate the nutritional properties of biscuit, methods published by the Association of the Official Analytical Chemists (1995) were used to measure the content of moisture, ash and mineral (Oh et al., 2016). Total dietary fibre (TDF) were estimated by enzymatic and gravimetric method using TDF-100 kit (Sigma) (Chen et al., 2014) in moisture and fat free samples. Total protein content in sample were determined by the semi-automatic Kjeldahl method according to AACC Method 46-12.01 (AACC, 2000) (Vujic, Cepo, and Dragojevic 2015). Total phenolic and total flavonoid content were evaluated through Folin–Ciocalteu assay (Lin et al., 2015) and aluminium nitrate method (Cheung, Cheung, and Ooi 2003) with some modifications. The caloric value of biscuits was determined by the method described by Lovorka (Vujic, Cepo, and Dragojevic 2015).

**Evaluation of microbial quality of olive pomace incorporated biscuits**

Microbial populations were estimated by serial dilution agar plating method (Seevaratnam et al., 2012). Briefly,
10 g biscuit was weighed in a sterile conical flask containing 90 mL of pre-warmed water. The sample was mixed thoroughly, diluted seriously (1:10). 1 mL of each of the dilution were then inoculated in sterile nutrient agar (for CFU detection), lactose bile medium (for *Escherichia coli* detection), rose bengal medium (for molds detection) and trypctase soy agar (for pathogenic bacteria detection), respectively. The plates were then incubated at 37 °C for 24 to 48 hours. The colony formation on agar plates were counted and convert into number of colony forming units (CFU) per gram of sample (Seevaratnam et al., 2012).

**Evaluation of in vitro starch digestibility and expected glycemic index**

The measurement of *in vitro* starch digestibility was carried out according to the method described previously (Goñi, Garcia-Alonso, and Saura-Calixto 1997; Ferrer-Mairal, Penalva-Lapuente, et al., 2012) with some modifications. Briefly, 1 g biscuit sample was dispersed in 9 mL H₂O. The mixture was placed in boiling water bath for 10 min and then cooled to room temperature before 1 mL HCl-KCl buffer (pH = 1.5) containing pepsin was added and incubated at 40 °C for 1 h. The sample were then treated with pancreatic α-amylase in Na₂HPO₄-NaH₂PO₄ buffer (pH 6.8) and incubated at 37 °C. 0.2 mL aliquot samples were taken from each tube at 0, 20, 40, 60, 80, 100, 120, 140, 160 and 180 and then immediately analyzed for reducing sugars. The glucose content was measured with a Glucose Quantitation Kit (Nanjing Jiancheng biological engineering research institute) and converted into starch by multiplying by 0.9. Commercial white bread was also analyzed as reference product.

A non-linear model used in previous publications (Ferrer-Mairal, Penalva-Lapuente, et al., 2012) was applied to describe the kinetics of starch hydrolysis and calculate expected GI:

The area under hydrolysis curve (AUC) was calculated according to equation 1:

\[
AUC = C_c(t_f-t_0) - \frac{(C_c/k)[1-e^{-k(t_f-t_0)}]}{t_f-t_0}
\]  

(1)

Here, \( C_c \) refers to the concentration at reaction equilibrium (\( t_{180} \)); \( t_f \) is the final reaction time (180 min); \( t_0 \) is the initial reaction time (0 min); and \( k \) value is the kinetic constant and calculated using equation 2:

\[
C = C_c \cdot [1-e^{-k\,t}]
\]  

(2)

where \( C \) is the concentration at \( t \) time, \( C_c \) is the concentration at reaction equilibrium (\( t_{180} \)), \( t \) is the chosen time

The expected glycemic index (eGI) was calculated using the equation 3:

\[
eGI = 0.549 \times \text{calcHI} + 39.71
\]  

(3)

Here, calcHI stands for calculated hydrolysis index, which was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained from white bread.

**Statistical analysis**

All experiments were performed at least three times. All data were presented as means ± standard error of the mean. As for multiple group comparison, the significance of the differences among the treatment groups and their respective control groups were analyzed using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was assessed by either Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison. Differences between means were considered statistically significant if \( p < 0.05 \).

**RESULTS**

**Characteristics of nutritional compositions of olive pomace**

The nutritional composition of the olive pomace is presented in Table 1. Consistent with previous reports (Clemente et al., 1997; Omer and Mohamed 2012), our results showed that olive pomace contains abundant dietary fibre. The total dietary fibre (TDF) accounted for around 80 % of the olive pomace composition with insoluble dietary fibre (IDF) representing the greatest part.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF/(%)</td>
<td>80.23±4.34</td>
</tr>
<tr>
<td>SDF/(%)</td>
<td>5.66±0.02</td>
</tr>
<tr>
<td>IDF/(%)</td>
<td>72.75±3.34</td>
</tr>
<tr>
<td>Protein/(%)</td>
<td>2.76±0.01</td>
</tr>
<tr>
<td>Ash/(%)</td>
<td>3.14±0.01</td>
</tr>
<tr>
<td>Moisture/(%)</td>
<td>6.25±0.02</td>
</tr>
<tr>
<td>Total Polyphenol/(%)</td>
<td>3.32±0.02</td>
</tr>
<tr>
<td>Total Flavone/(%)</td>
<td>0.83±0.03</td>
</tr>
<tr>
<td>Ca/(mg/kg)</td>
<td>731.15±5.32</td>
</tr>
<tr>
<td>Mg/(mg/kg)</td>
<td>301.16±3.45</td>
</tr>
<tr>
<td>Na/(mg/kg)</td>
<td>27.91±2.98</td>
</tr>
<tr>
<td>Fe/(mg/kg)</td>
<td>23.42±0.73</td>
</tr>
<tr>
<td>Mn/(mg/kg)</td>
<td>12.53±0.22</td>
</tr>
<tr>
<td>Zn/(mg/kg)</td>
<td>8.87±0.15</td>
</tr>
<tr>
<td>Cu/(mg/kg)</td>
<td>4.25±0.06</td>
</tr>
<tr>
<td>Cr/(mg/kg)</td>
<td>1.45±0.02</td>
</tr>
<tr>
<td>Co/(mg/kg)</td>
<td>0.86±0.02</td>
</tr>
<tr>
<td>Ni/(mg/kg)</td>
<td>0.41±0.01</td>
</tr>
</tbody>
</table>

Results are reported as means±SD of three investigated series of olive pomace (n=3)
The moisture, ash and protein content of olive pomace recorded in this study was 6.25 (±0.02) %, 3.14 (± 0.01) % and 2.76 (± 0.01) % respectively, which was also comparable to previous studies (Clemente et al., 1997; Omer and Mohamed 2012). In addition, the olive pomace also has approximate total polyphenol and total flavone contents of 3.32 (±0.02) % and 0.83 (±0.03) %, respectively; while it is also rich in minerals such as calcium (731.15 (±5.32) mg/kg) and magnesium (301.16 (±3.45) mg/kg). Taken together, these results showed olive pomace is abundant source of dietary fibre with high polyphenol, flavone, and minerals components, and may be developed as a supplementary ingredient for making high dietary fibre biscuit.

Preparation of olive pomace incorporated biscuit

Firstly, the levels of olive pomace incorporation were optimized according to the sensory characteristics and acceptability of the biscuits. As shown in Table 2, majority of tested sensory attributes received comparable scores between olive pomace incorporated biscuits and control biscuits, except significant drops in taste, texture/doneness, and overall acceptability were observed in biscuit with olive pomace replacing wheat flour at level of 20 %. Moreover, the biscuits with 15 % incorporation level received highest overall acceptability with score of 4.58(±0.50). The biscuits with this recipe also received highest scores in other sensory attributes including color, appearance, flavor, taste and texture/doneness among all tested levels of olive pomace incorporation, suggesting incorporating olive pomace powder as wheat flour replacers up to a level of 15 % could be well accepted. Indeed, previous study has demonstrated that biscuits incorporated with non-digestible oligosaccharides were acceptable to the consumer and reached commercial acceptability (Boobier, Baker, and Davies 2006), also highlighting the satisfactory acceptance of high-fibre biscuits. Therefore, according to the sensory properties evaluation, the recipe for the biscuit dough was chosen as 85 g wheat flour powder, 15 g olive pomace powder, 33 g sugar, 22 g shortening, 1.1 g sodium chloride, 0.7 g sodium bicarbonate, 0.3 g ammonium bicarbonate and 6 g skimmed milk powder, one egg (50-56 g) and 12.5 mL water. Indicative pictures of produced biscuits are presented in Supplementary Fig. 1.

Nutritional properties and microbial quality of Olive pomace incorporated biscuit

The nutritional composition and microbiological quality of the modified biscuits were next investigated. As expected, the olive pomace-based biscuits contain significantly more dietary fibre than the traditional wheat flour biscuits. Meanwhile, caloric value of olive pomace-based biscuits (454.2 (±0.77) kcal/100 g) also reached a small, but statistically significant reduction in comparison to the control (469.09 (±0.65) kcal/100 g) as shown in Table 3. Besides, the results also demonstrated the protein and fat content are comparable in both investigated biscuits. Our results also revealed that the nutritive values and the microbial quality of both levels of olive pomace incorporation were statistically significant (p<0.05).

Table 2: Mean sensory scores of different treatments of Chinese olive pomace based high fibre biscuits

<table>
<thead>
<tr>
<th>Levels of olive pomace incorporation as wheat flour replacers</th>
<th>Color</th>
<th>Appearance</th>
<th>Flavor</th>
<th>Taste</th>
<th>Texture/Doneness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.40±0.81a</td>
<td>4.62±0.60a</td>
<td>4.58±0.57a</td>
<td>4.58±0.53a</td>
<td>4.54±0.54a</td>
<td>4.52±0.61a</td>
</tr>
<tr>
<td>10%</td>
<td>4.26±0.99b</td>
<td>4.58±0.57b</td>
<td>4.40±0.90b</td>
<td>4.54±0.54b</td>
<td>4.52±0.58b</td>
<td>4.54±0.54b</td>
</tr>
<tr>
<td>15%</td>
<td>4.68±0.47b</td>
<td>4.66±0.48b</td>
<td>4.44±0.81b</td>
<td>4.60±0.53b</td>
<td>4.56±0.54b</td>
<td>4.58±0.50b</td>
</tr>
<tr>
<td>20%</td>
<td>4.48±0.81a</td>
<td>4.58±0.70a</td>
<td>4.28±0.99a</td>
<td>4.26±0.63a</td>
<td>4.16±0.77a</td>
<td>4.10±1.04a</td>
</tr>
</tbody>
</table>

Results are reported as means±SD of three investigated series of olive pomace (n=3), data in the same column marked with different letter are statistically significant (p<0.05)

Table 3: Nutritional properties and microbial quality of biscuits

<table>
<thead>
<tr>
<th>Items</th>
<th>Olive pomace based biscuits</th>
<th>Traditional wheat flour biscuits</th>
<th>National standard of china</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, g/100g of original sample</td>
<td>3.05±0.45a</td>
<td>2.60±0.26a</td>
<td>≤4.0</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.82±0.13b</td>
<td>1.95±0.29b</td>
<td></td>
</tr>
<tr>
<td>Fat, g/100 g of original sample</td>
<td>23.30±0.43a</td>
<td>24.20±0.76a</td>
<td></td>
</tr>
<tr>
<td>Protein, g/100 g of original sample</td>
<td>8.20±0.26a</td>
<td>7.10±0.31a</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates, g/100 g of original sample</td>
<td>32.50±0.56a</td>
<td>50.2±0.73a</td>
<td></td>
</tr>
<tr>
<td>Energy (Kcal)/100 g of original sample</td>
<td>454.20±1.77a</td>
<td>469.09±2.65a</td>
<td></td>
</tr>
<tr>
<td>Dietary fibre, g/100 g of original sample</td>
<td>10.20±0.66a</td>
<td>1.1±0.17a</td>
<td></td>
</tr>
<tr>
<td>Colony count, cfu/g</td>
<td>9.3±4.9a</td>
<td>8.0±3.0a</td>
<td>≤750</td>
</tr>
<tr>
<td>Escherichia coli, MPN/100g</td>
<td>3.0±1.0a</td>
<td>3.3±0.6a</td>
<td>≤30</td>
</tr>
<tr>
<td>Molds, cfu/g</td>
<td>0.6±0.5a</td>
<td>1.6±0.6a</td>
<td>≤50</td>
</tr>
<tr>
<td>Pathogenic bacteria</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Results are reported as means±SD of three investigated series of biscuits (n=3), Data in the same row marked with different letter are statistically significant (p<0.05)
biscuits conformed to the current national standard of China (GB5009.56-2003), suggesting the incorporation of olive pomace will not increase microbial contamination risk. Previous study also demonstrated a similar results in the microbial examination of dietary fiber incorporated biscuits (Seevaratnam et al., 2012). Taken together, the results indicated that the olive pomace based biscuit is a fibre-rich functional food with high nutritional quality and microbial safety.

Starch digestibility of olive pomace incorporated biscuit

Dietary fiber, as the indigestible portion of food, is resistant to digestion and absorption in gastrointestinal tract (Otles and Özgoz 2014). Previous works have shown that high-fibre food led to the lower starch hydrolysis in vitro (Leoro et al., 2010; David Barine and Yorte 2016), therefore, it is expected that the incorporation of olive pomace may reduce the starch digestibility and consequently expected GI in vitro. As shown in Fig. 1, the olive pomace incorporated biscuit released less glucose in first 20 mins after addition of α-amylase (representing rapidly available glucose in vivo) than control biscuit. The difference in released glucose was more obvious after 120 min α-amylase digestion (reflecting slowly available glucose in vivo). Table 4 also reflected this trend that the biscuits with partial replacement of wheat flour by olive pomace powder demonstrated significant reduced calcHI (43.66±0.58 vs 67.31±0.75) comparing with the control biscuits. The expected GI (eGI) also demonstrated a similar trend between these two types of biscuits (63.68±1.23 vs. 76.66±2.25). Furthermore, since previous study has suggested that the starch fraction remaining undigested after 120 min correlates with the content of resistant starch in investigated sample. Here the starch digestibility results were also in accordance with the significant increase in the dietary fibre content in olive pomace incorporated biscuit.

**DISCUSSION**

This study was conducted to establish an optimized proportion of refined wheat flour powder and olive pomace to formulate the high dietary fibre biscuit. The results showed olive pomace powder could be incorporated up to 15% level without significant changes of sensory characteristics. Moreover, olive pomace incorporation improved the nutritional properties of new formulated biscuits, especially a significant increase in dietary fibre content was observed. The calculate starch hydrolysis index and expected glycemic index of this olive pomace based biscuits were also significant lower than traditional wheat flour biscuits. Previous studies have pointed out that despite the expected GI in vitro may not fully reflect the GI measured in vivo, however, the trend toward GI reduction seems to be similar for both in vitro and in vivo methods (Konstantina et al., 2016; Vujic, Cepo, and Dragojevic 2015; Lai et al., 2016; Ferrer-Mairal, Peñalva-Lapuente, et al., 2012), especially when take into the consideration that both biscuits owned similar physical form. Indeed, previous studies also revealed that incorporation of high fibre ingredient in bakery production could be a promising strategy to significantly decrease the GI in vivo. For example, it was reported that substituting the wheat flour with dietary fiber-rich pulse ingredients could reduce the GI value of cereal-based products in vivo (Fujikawa, Hall, and Jenkins 2016). Besides, Ferrer-Mairal also demonstrated that a reduction of GI value in the in vitro and in vivo was achieved with the partial substitution of wheat flour by dietary in muffin and breads (Ferrer-Mairal, Peñalva-Lapuente, et al., 2012). Therefore, it is highly likely that olive pomace incorporation may also lower the GI of this new formulated biscuit in vivo. Considering the beneficial effects of lower glycemic index (GI) diet on human health such as decreasing postprandial glycaemia and insulin levels, improving blood glucose control, and preventing the cardiovascular diseases and diabetes mellitus (Augustin et al., 2002), the receipt established in this study might be used to produce the biscuits as a healthy bakery snack to increase the intake of dietary fibre (Roberts 2000).

**CONCLUSION**

In conclusion, the current study demonstrated the olive pomace could be used as a potential ingredient for high-fibre biscuits production. Since the high-fibre recipes have become a trend in the bakery industry, the use of olive pomace may also be exploited successfully in other bakery...
products. In the future, the development of new foods based on olive pomace may also have a wide application prospects.

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Authors’ contributions

Conceived and designed the experiments: SL, BZ, SZ. Performed the experiments: WC, QP. Analysed the data: SL, WC, JH. Contributed reagents/materials/analysis tools: SL, BZ, SZ. Prepared the paper: SL, JH.

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