Enhancing the properties and oxidative stability of margarine through *Moringa* oil enrichment

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Reports on moringa oil being a great food source. Because of the high nutritional content and possible health benefits associated with its chemical makeup, moringa oil is categorised as a nutraceutical food. This study aimed to valorise *Moringa oleifera oil* and evaluate the effect of its incorporation at different concentrations on the properties and oxidative stability of margarine during a one-month refrigerated storage.

Moringa oleifera seeds were extracted in a cold press, which have undergone preparation operations and degreased by passing through a continuous-feed screw press (horizontal press) of the Xeoleo screw press. The Moringa oil was incorporated into margarine at the following two levels: 5% (M05%) and 15% (M015%).

The best way to preserve the high biochemical quality of MO, with good concentrations of fatty acids and triglycerides, particularly oleic acid, was to extract the oil by cold pressing. MO extracted for this investigation has high levels of β -sitosterol (49.85%) and α -tocopherol (243.02 mg/kg). The results showed that this oil presented respective peroxide value, acidity and iodine index of about 2.47 meq O₂/Kg, 0.84 mg KOH/g and 64.53 g/100g, with good contents of polyphenols (0.163 mg/kg) and carotenoids (3.26 mg/kg). Additionally, the efficacy of including MO in high-fat diets was validated by preventing margarine's oxidative stability while it was refrigerated in comparison to the control. The strong antioxidant activity of the recently created margarine qualifies it as a functional product. Nonetheless, significant water content was the outcome of the 15% moringa oil dosage.

Moringa oil is extremely resistant to autoxidation and can be used as an antioxidant for the long-term stabilisation of commercial edible oils.

Keywords: Moringa oleifera; oil; antioxidant; margarine; durability of oxidation

1. INTRODUCTION

Moringa oleifera (MO) is a member of the Moringaceae family, cultivated worldwide for its interesting numerous properties including nutritional, medical and industrial potential [1]. All parts of the MO tree have good nutritional and therapeutic values [2]. Proteins, minerals, β -carotene, and naturally occurring antioxidants are abundant in the leaves. They are used not only for human and animal nutrition, but also in traditional medicine [3]. On the other hand, MO seeds have been used to cure or prevent inflammation, rheumatism, bacterial and fungal infections, constipation, arthritis and hypertension [4]. In addition, the seed is a source of protein, minerals such as zinc and magnesium, oleic acid, as well as antioxidants [5]. Because of its qualities, this oil can be used for cosmetic and pharmacological applications as well as for human food as well as non-food applications, such as biodiesel, cosmetics and a lubricant for fine machinery

Salama et al. (2018) [6] revealed that MO greatly increased the content of fatty acids (oleic acid > 70%) as well as triglycerides (triolein > 30%). Gharsallah et al. [7] state that β -sitosterol and α -tocopherol are the two most compounds.

Today's consumers are increasingly concerned about food quality and their nutritional attributes. To meet consumer needs and create functional foods that are beneficial to human health, it is now necessary to enrich foods with nutrients to improve their nutritional content. Due to its widespread commercialisation, lower cost, use in the bakery and confectionery industries, and seasonal independence, margarine is currently experiencing a global market expansion [8]. trans-fatty acids are being phased out of industrial margarines by the World Health Organisation due to their established link to an increased risk of cardiovascular disease. To accomplish this, producers are currently working to create new margarines that include non-lipid ingredients and additional phytosterols to guarantee a nutritious and useful product [8]. However, oxidation is one of the most widespread phenomena in the food industry particularly in fat-rich products. Among these goods, margarine is a good illustration of one that is oxidation-prone because it contains 80% fat [9]. The aim of this work was to evaluate if MO oil might be a novel antioxidant for stability and preservation in the oxidation of margarine. Hence, two amounts of 5% and 15% of this oil was incorporated in the margarine, after a month of storage at +7°C, the physicochemical parameters, including oxidative stability and antioxidant activity, were assessed.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL AND MORINGA OIL EXTRACTION The seeds of *Moringa* were harvested just after ripening from the Chams Industrial (Sidi Thabet, Ariana) during 2021. The seeds were extracted in a cold press, which have been degreased by being inserted through a continuous-feed screw press of the Xeoleo screw. Centrifugation at 5000 rpm for 15 min is used after sieving to extract the oil.

2.2. FEATURES OF PHYSIOCHEMISTRY OF MORINGA OLEIFERA OIL

The official protocols were followed to determine the extracted MO's acid, peroxide, iodine, saponification, refractive at 20°C, and specific absorptivity values K232 et K270 [10]. Using the hunter L*, a*, and b*, a Chromameter (Konika Minolta, Sensing INC, Japan) measured the surface colour of the cookie samples under study. Black (0) to white (100) is measured by the L* values, and a* values quantity (+100) and greenness (-100), and b* values measure yellowness (+100) and blueness (-100). Chlorophyll and carotenoid compounds were determined in oil samples according to the method described by Haddada et al. [11] using a by a Cary 60 UV-vis spectrophotometer at wavelengths of 470 nm and 670 nm, respectively.

2.3. FATTY ACID AND TRIACYLGLYCEROL ANALYSIS

The composition of fatty acids was performed using gas chromatography (GC/FID) according to the method described in the European Commission Regulation (EU No 2015/1833). After the transesterification of the fatty acids, the obtained fatty acid methyl esters (FAMEs) were analysed using GC (Agilent system 7890A) with a capillary column HP-5MS $(30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ }\mu\text{m})$. The separation was involved with a programmed temperature (110 °C held for 5 min, increase of 3 °C/min to 150 °C and held for 16.33 min, increase of 4 °C /min to 230 °C and held for 27 min) and the flame ionisation detector (FID) temperature was 150 °C. FAs were identified by comparing retention times to standard compounds. To produce the triacylglycerol (TAG) profile, high-performance liquid chromatography (HPLC) equipped with a reversed-phase C18 column (250 x 4.5 mm and 5 µm particle size) was used according to the International Olive Council. (2010).

2.4. STEROL AND TOCOPHEROL ANALYSIS

The International Olive Oil Council's standard technique was used to determine the sterol composition. The silylated sterol fraction was separated and quantified using capillary gas chromatography (GC2010, Shimadzu, Japan) with a flame ionisation detector (FID) and a Supelco (SPBTM-5 24034, Bellefonte, USA) capillary column (30 m, 0.25 mm, i.d. and 0.25 mm film thickness). The temperature in the column was 260°C. The temperatures of the injector and detector were 280°C and 290°C, respectively. A split ratio of 50:1 was employed with helium serving as the carrier gas, with a flow rate of 1 ml/min.

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Tocopherol extraction and analysis were determined according to the method described in the International Olive Council. (2010) using a high-performance liquid chromatography (HPLC) equipped with Agilent HP1100 (Agilent Technologies, Palo Alto, CA, USA).

2.5. QUANTIFICATION OF CONDENSED TANNIN (CT), TOTAL FLAVOINOIDS (TF), AND TOTAL POLYPHENOLS (TP)

CT was calculated using the method described by Salar and Purewal's [13]. The amount of CT was expressed as mg catechin equivalent CE/g oil. To determine TF, the method of Páramo-Calderón et al. [14] was used. Results were expressed as mg quercetin equivalent QE/g oil. TP was estimated using Folin-Ciocalteu colorimetric method [14]. Results were expressed as mg gallic acid equivalent GA/g oil.

2.6. ANTIRADICAL ACTION

Ceylan et al. [15] approach was utilised to measure the radical scavenging activities of MO. Three millilitres of a DPPH solution (0.004% in methanol) were combined with half a millilitre of samples at varying concentrations in microtubes. After 30 min of standing at room temperature in the dark, the mixture's absorbance was measured at 517 nm. The DPPH free radical's inhibition (lh%) was calculated using the following formula:

lh% = [(absorbance of the control- absorbance of the trial)/ absorbance of the control]×100, The concentration of extract (IC50) that could scavenge 50% of the DPPH radicals was determined.

2.7. MARGARINE SAMPLES PREPARATIONS

To study the effect of the incorporation of MO on the quality of commercial margarine (Goldina), purchased from a local market, an amount of 5% and 15% of this oil was added. The integration of MO in margarine was made according to the procedure outlined by Nadeem et al. [16]. The physicochemical properties (moisture, acidity, refractive index and peroxide value) and the evolution of the antioxidant activity were evaluated for the formulated and control margarines, after a month of preservation at $+7^{\circ}$ C.

2.8. ANALYTICAL STATISTICS

Every extraction and calculation were carried out three times. The mean and standard deviation (SD) of the data are expressed. Using SPSS 23.0 (SPSS IBM2017), an analysis of variance (ANOVA) was conducted.

3. RESULTS AND DISCUSSION

3.1. PHYSICOCHEMICAL CHRACTERISATION OF MO

Our oil was classified as non-siccative with an index of refraction (IR) that is 1.465 (Table 1). This finding is nearly comparable to Gharsallah et al. [7] that reported that the IR from the MO was 1.462. The cold press MO's acid index is 0.848 mg KOH/g oil. The results showed that a peroxide index of 2.47 meq O2/Kg. This value is lower than required by the Codex Alimentarius. Khemakhem et al. [17], were recorded that the peroxide ranging from 3.3 to 4.5 meq O2/Kg in oil of pomegranate seeds.

The measured saponification index for the MO is 188 mg KOH/g oil, which is comparable to the olive oil that is between 184 and 196 mg KOH/g oil. The saponification value for use as an alternative fuel oil is 183.20 mg KOH/kg. This is within the range of 170-195 mg KOH/kg recommended in literature [18].

The iodine value is 64.53 g/100g. Our result is lower than that found by Gharsallah et al. [7] reporting 67.42 g/100g. However, the value found is lower than that of olive oil which varies between 75 and 94 g/100g. Our sample's K270 and K232, are lower, coming in at 0.0576 and 1.2345, respectively (Table 1). These results indicate that secondary oxidation is limited by the natural antioxidants in our oil. The results obtained are comparable to those found by Gharsallah et al. [7].

3.2. COMPOSITION OF CHLOROPHYLL AND CAROTENOID OF MO

The results showed that the carotenoid content of MO studied is 3.26 mg/kg (Table 1). According to Zhuang et al. [19], carotenoid levels depend on the degree of maturation and their protective role against oxidation. Conversely, the chlorophyll content is 1.56 mg/kg. Based on studies by Conesa et al. [20] the content of chlorophyll is negligible and almost absent for MO oil. It should be noted that chlorophyll and carotenoid give the extracted oil the green and yellow coloration,

Table I - Physiochemical characteristics of Moringa oleifera oil

Proventing.	Mahara
Properties	values
Oil yield (%)	14±0.87
Refractive index (20°C)	1.46±0.02
Acide value (mg kOH/g oil)	0.84±0.03
Peroxide value (meq O ₂ /kg oil)	2.47±0.32
Saponification value (mg KOH/g oil)	188±2.14
lodine value (g l2/100g oil)	64.53±0.25
K232	1.23±0.02
K270	0.057±0.01
Color	
L*	98.49±0.03
a*	-1.44±1.07
b*	58.64±4.5
Carotenoid (mg/Kg)	3.26±0.12
Chlorophyll (mg/Kg)	1.56±0.03
Total polyphenol (mg GA/g oil)	0.163±0.01
Total Flavonoid (mg QE/g oil)	0.355±0.03
Condensed tannin (mg CE/g oil)	0.041±0.5
IC ₅₀ (μg/ml)	81±1.23

K232 and K270: Specific extinctions coefficients at 232 and 270 nm, Values are means ±standards deviations.

respectively, and that their contents depend mainly on the seed maturity.

Table 1 showed that the brightness L* of MO is 98.49 which indicates that our oil is quite clear. Regarding the parameter a*, we recorded a negative value of -1.44 highlighting a colour showing the colour of MO changing to green. As for parameter b*, the results showed a higher value of the yellow colouration (58.64). These values are partially consistent with those recorded by Gharsallah et al. [7]. Indeed, it has been reported that the major pigments of oils obtained by cold press are carotenoids and chlorophylls [7].

3.3. TP, TF AND CT

TP is 0.163 mg AG/g oil, as shown in Table 1. This level is higher than what was discovered by Gharsallah et al. [7], who reported a TPC of 0.102 mg AG/g oil. However, this level is lower than that recorded for olive oil, which was 0.32 mg EAG/g oil [21].

It should be noted that the TP in the oil is considered a natural antioxidant that prevents oxidation and provides a better storage stability, bitter flavour and pungency [22]. TP also prevents cardiovascular disease and cancer [7] and this encourages its incorporation in food products.

The TF in MO is 0.355 mg QE/g oil (Table 1). In fact, this level is higher than olive oil (0.174 mg EC/g oil) [21].

Based on Table 1, the CT in MO is 0.041 mg CE/g oil. The presence of tannins suggests the ability of our plant to play an important role as an antimicrobial and antioxidant.

The IC50 is 81 µg/mL (Table 1). Similarly, our recorded value is higher than that found by Bhatnagar and Krishna [23] who reported an IC50 of 35.5 µg/ml of MO from India. It is evident from the IC50 value that MO is among the oils with the highest concentration of naturally occurring antioxidants.

3.4. COMPOSITION OF FATTY ACID, TOCOPHEROL, STEROL AND TRIGGLYCERIDES PROFILES

Eleven fatty acids were identified where oleic acid was predominant (82.32%) (Table 2). These findings bear some resemblance to those of Gharsallah et al. [7], where oleic acid (73.36%) was predominant. Because of its high oleic acid content, MO has a higher nutritional value and is more palatable due to its superior holding and stability during heating and frying. In fact, this oil oxidises less than other oils rich in polyunsaturated fatty acids. Besides, the saturated fatty acid content did not exceed 15% (Table 2).

According to the findings, the sterol fraction of MO was β -sitosterol (49.85%), stigmasterol (25.74%), campesterol (23.15%) representing 90% of total

Fatty acids (%)		Sterols (%)		Triacylglycerol (TAG,%)		Tocopherols (mg/kg oil)	
Myristic acid C14:0	0.10±0.01	Cholesterol	0.10±0.02	000	39.21±1.23	a-Tocopherol	243.02±2.14
Palmitic acid C16:0	5.90±0.03	Stigmasterol	25.74±1.23	P00	13.53±1.03	y-Tocopherol	112.7±1.26
Palmitoleic acid C16:1n-7	1.24±0.04	Campesterol	23.15±1.45	S00	11.28±1.11	δ-Tocopherol	11.7±0.55
Heptadecanoic acid C17: 0	0.05±0.01	β-Sitosterol	49.85±2.31	OOA	7.39±0.45		
Ginkgolic acid C17: 1	0.03±0.00	Chlerosterol	0.40±0.05	POL	4.8±0.07		
Stearic acid C18:0	5.28±0.02	Δ ⁷ -Avenasterol	0.29±0.02	PLS	2.48±0.04		
Oleic acid C18:1n-9	82.32±1.82	Δ ⁵ ,23 Stigmastadienol	0.27±0.03	POS+SLS	2.37±0.03		
Linoleic acid C18:2n-6	0.68±0.03	Δ ⁷ -Stigmastenol	0.20±0.02	SOS	2.11±0.05		
α-Linolenic acid C18:3n−3	0.13±0.01	Erythrodiol	0.10±0.01	LLL	0.31±0.01		
Arachidic acid C20:0	2.98±0.02	Uvaol	0.04±0.01	OLLn	0.18±0.00		
Gadoleic acid C20:1n-9	1.30±0.01			PLLn	0.16±0.01		
ΣSFA	14±0.56			Others	16.83±1.21		
ΣMUFA	84.89±1.35						
ΣPUFA	0.81±0.03						
Ratio unsaturated / saturated	6.12						

Table II - Fatty acids, sterols, triacylglycerols and tocopherols composition of Moringa oleifera oil

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. P: radical palmityl. PO: radical palmitoleyl. O: radical oleyl.

L: linoleyl radical. Ln: linolenyl radical. S: stearyl radical. All measurements were done in triplicate and results were expressed as means ± SD.

sterols (Table 2). These sterols are known for their cholesterol-lowering effect and health-promoting effects [24].

These interesting results highlight the oil's high nutritional content and pertain to the examination of cold-pressed MO. The outcomes obtained allowed that α -tocopherol is predominant of 243.02 mg/kg oil followed by γ -tocopherol (112.7 mg/kg oil) and δ -tocopherol (11.7 mg/kg of oil) (Table 2). The content of α -tocopherol in MO agreed with those reported for soybean, groundnut and palm oils. These values were higher than those detected by Pluháčková et al. [25]. Literature revealed that α -isomer of tocopherol has a greater vitamin E potency, whereas δ -isomer of tocopherol has a greater of the source of the sourc

As shown in Table 2, twelve triglycerides were detected mainly OOO (39.21%), POO (13.53%) and SOO (11.28%). Our results are consistent with those of Salama et al. [6] who highlighted that the most TAG was OOO (34.81%), followed by OOP (14.50%) and SOO (11.25%). The analysis of TAG allowed confirming our results of fatty acids.

3.5. EFFECT OF MO INCORPORATION ON MARGARINE

3.5.1 Moisture, acidity and refractive index

The moisture of the margarine (MC), as shown in Table 3, is 6.10%. Thus, the water content of the MC and those supplemented with MO did not exceed the standard required by the Codex Alimentarius, who emphasised that the content should be between 16% and 18%. After 15 days of storage, the water content of MC and MO5% increased to 9.54% and 10.037%, respectively, without exceeding the norm. In return, margarine with 15% of MO (MO15%) was higher in in water (13.64%) showing that the incorporation of a high concentration of MO induced a significant increase (p<0.05) during the conservation.

Silva et al. [8] emphasised that the water content of margarine is a parameter that affects its physical characteristics without having a major impact on its stability. Therefore, the addition of MO5% showed a water content the closest to that of the control with a significant difference (p<0.05) throughout the time spent refrigerated.

Every sample that was examined revealed a steady rise in acidity over time while being stored (Table 3). After 30 days, the control's acidity rose from its initial value of 0.14% to 0.18%. Acidity measured for MC, MO5% and MO15% were 0.14%, 0.13% and 0.12% respectively (Table 3). Our results are higher than those recorded by Nadeem and Imran [16] reporting initial values not exceeding 0.11%. Following the conservation, we took notes on acidities in the order of MC (0.18%), MO5% (0.15%) and MO15% (0.14%). These values did not exceed the limit value (0.2%) recommended by Nadeem et al. [16]. This result showed that higher the dose of MO, the more the acidity decreases confirming our findings on the antioxidant activity of this oil due to its richness in polyphenols and some pigments [23].

From Table 3, the refractive index increases with the dose of MO. Initially, the refractive index was 1.496 (MC) and 1.499 (MO15%). These results are in line with those of Nadeem et al. [16]. This index decreased significantly for all margarines analysed over 30 days of storage. However, no significant difference (p<0.05) was observed between the control and fortified margarines throughout their shelf life.

3.5.2 Peroxide index

Initially, MO15% had the lowest peroxide value measured, 0.3 O2/Kg (Figure 1). All initial PI values are lower than the standard (5 O2/kg). This result confirms the antioxidant activity of MO and its effectiveness in improving oxidative stability in the preservation of highfat foods such as margarine [23]. In fact, margarines

Storage period (days)	Samples	Moisture (%)	Acidity (%)	Refractive index
0	MC	6.10±0.001 ^{aA}	0.14±0.01 ^{aA}	1.49±0.002 ^{aA}
	MO5%	7.37±0.003 ^{bA}	0.13±0.02 ^{bA}	1.49±0.002 ^{aA}
	MO15%	9.30±0.005 ^{cA}	0.12±0.01 ^{cA}	1.49±0.003 ^{aA}
15	MC	9.54±0.02 ^{aB}	0.16±0.01 ^{aB}	1.49±0.01 ^{aA}
	MO5%	10.03±0.001 ^{aB}	0.14±0.02 ^{aB}	1.49±0.02ªA
	MO15%	13.64±0.05 ^{bB}	0.13±0.03 ^{bB}	1.49±0.01 ^{aA}
30	MC	13.88±0.03 ^{aC}	0.18±0.01 ^{aB}	1.49±0.001 ^{aA}
	MO5%	15.81±0.05 ^{bC}	0.15±0.02 ^{bB}	1.49±0.003 ^{aA}
	MO15%	18.51±0.01 ^{cC}	0.14±0.02 ^{cC}	1.49±0.002 ^{aA}

 Table III - Physicochemical composition of margarine enriched with Moringa oleifera oil during a refrigerated storage

MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added. All measurements were done in triplicate and results were expressed as means \pm SD. Values followed different letters (a-c) in the same column indicated significant differences by the Duncan test different at P < 0.05.

enriched with MO were more resistant to peroxidation compared to the control. Indeed, MO15% had better oxidation resistance than MO5%. The outcomes are logical since our oil showed an important IC50 of 81 µg/ml. However, the PI values found are lower than those reported by Silva et al. [8]. These results show that MO can be used to improve the oxidative stability of commercial edible oils [16].

3.5.3 Inhibition percentage

The DPPH activity was found to be 81 μ g/ml, indicating that forid-pressed is a highly potent natural antioxidant. It has a powerful DPPH°-neutralising capacity at low doses.

A significant difference was observed between the different margarine samples analysed (Figure 2). In fact, the best percentage inhibition (35.25%) was noted for margarine MO15%. This parameter underwent a

significant increase (p<0.05) during refrigerated margarine storage. In fact, the inhibition percentage of margarine (MC) increased from 26.47% to attain a value of 42.52%. As for the MO 5% sample, a change in this parameter was noted, after 30 days, from 31.53% to 49.45%. Also, the best inhibition (57.56%) was recorded for the MH15 margarine, after being kept at 7°C for 30 days.

These findings corroborated those of the peroxide indices, which showed that even at modest dosages (5%), the inclusion of MO enhanced the margarine's antioxidant ability.

3.5.4 Colour

Changes in the colour parameters L* (lightness), a* (redness) and b* (yellowness) of all MO during storage are illustrated in Figure 3. The colour L* varied significantly during storage after treatment with MO. For the



Figure 1 - Evolution of the peroxide value of control and enriched margarines during one month of refrigerated storage. MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added



Figure 2 - Percentage change in inhibition of control and enriched margarines during one month of refrigerated storage. MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added. All measurements were done in triplicate and results were expressed as means ± SD. Values followed different letters (a-c) in the same column indicated significant differences by the Duncan test different at P < 0.05.



Figure 3 - Changes in color parameters of control and enriched margarines during one month of refrigerated storage. MC: Control margarine; MO5%: Margarine with 5% *Moringa oleifera* oil added; MO15%: Margarine with 15% *Moringa oleifera* oil added. All measurements were done in triplicate and results were expressed as means ± SD. Values followed different letters (a-b) in the same column indicated significant differences by the Duncan test different at P < 0.05.

untreated MC, the value of L* decreased slightly with the dose of MO during storage. In fact, the luminosity of the control and fortified margarines decreased during storage. It was observed that the value of b* increases with the increasing concentration of MO (Figure 3). In fact, the typical colour of margarines is due to the richness of MO in carotenoids. After 30 days, the highest b* (18.467) was found for MO15%. This result is due to the oxidation of the carotenoids, which leads to a decrease in the yellow colour of the margarine. MO is extremely resistant to autoxidation and can be used as an antioxidant for the long-term stabilisation of commercial edible oils. MO's high oleic content may have the ability to increase beneficial HDL cholesterol and decrease serum cholesterol and triglycerides [16].

4. CONCLUSION

During storage, margarine with MO15% showed better resistance to oxidation, with the lowest peroxide value and the highest inhibition percentage, due to the high content of TP in MO, which are very powerful natural antioxidants. These results were also confirmed by the determination of acidity, which was the lowest at 0.14% in MO15%. However, this same enriched margarine showed a high-water content. The findings indicated that the high carotenoid content of the MO contributed to an increase of the yellow hue with an increasing added concentration. Similarly, the colour parameter b* decreased during storage in all the margarines analysed, due to the oxidation of the carotenoids responsible for the typical colour.

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