



ORIGINAL ARTICLE

Comparative study of total phenolic content and antioxidant properties of Quercus fruit: flour and oil

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Abstract

Aim: The current study was undertaken to determine the total phenolic and flavonoid contents and to assess the antioxidant activity of two different extracts (flour and oil) of two Algerian *Quercus* species, *Quercus ilex* L. and *Quercus suber* L. **Methods and Material:** The oil extraction of the two species was achieved using the Soxhlet method. The obtained extracts were estimated for the chemical and physical constants (acidity, peroxide value, iodine value, and ultraviolet absorption indices). Total phenolic content was measured by spectrophotometry according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalents (GAE). The studied extracts were submitted to an estimation of their flavonoid contents too, using aluminum chloride methods. Antioxidant ability was assessed by means of two distinct methods (DPPH• and ABTS•+). **Results:** The obtained results revealed that antioxidant properties, total phenolic, and total flavonoid contents differed significantly among selected species and extracts. The flour samples possessed the highest level of total phenolic contents (1101–1464 mg GAE/kg dry weight) and exhibited the highest antioxidant capacities with average values of 52.62–40.78 μmol TE g⁻¹ dry weight and 36.19–44.50 μmol TE g⁻¹ dry weight for DPPH and ABTS assays, respectively. Acorn oil extracts showed also remarkable antioxidant activity, up to 2.69 and 3.23 μmol TE g⁻¹ oil (DPPH and ABTS test, respectively), even though the total phenolic contents were low (195.64–322.06 mg GAE /kg of oil). Total phenolic amounts were positively correlated with the antioxidant properties of *Quercus* flour and oil. **Conclusions:** Our study provides basic information on the presence of bioactive compounds and antioxidant capacity in acorn fruits, in order to consider their extracts as functional food ingredients and potential source of natural antioxidants.

Keywords: *Quercus ilex* L., *Quercus suber* L., Total phenolic, Total flavonoid, Antioxidant activity.

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1 Introduction

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant system [1-3]. The ROS are constituted of free radicals (like OH•, O₂•⁻, RO₂•) and non-radical products such as H₂O₂, RO₂H) [4,5]. While the defense system against free radicals can be divided into two major groups: enzymatic antioxidants' group, including superoxide dismutase, glutathione peroxidase, and glutathione reductase. The non-enzymatic antioxidants that constitute the second group consist of vitamins E and C, carotenoids, and polyphenols [6,7].

Phenolic compounds are one of the most essential groups, among phytochemicals possessing antioxidant capacity [8-10]. The designation "phenolic compounds" is generic referring to a large number of compounds (more than 8,000) widely dispersed throughout the plant kingdom [8,10,11]. Phenolics

are produced in plants as secondary metabolites. Their antioxidant activity is related to their chemical structure that provide them redox properties. They can play a significant role in neutralizing reactive oxygen species, quenching singlet and triplet oxygen, or decomposing peroxides [8]. Owing to their potential health-promoting effects, phenolic compounds have been intensively investigated [12,13]. Indeed, they are involved in the prevention and treatment of several diseases linked to oxidative stress such as cancers, atherosclerosis, diabetes, inflammation, and neurodegenerative diseases [8,11,13,14]. Furthermore, phenolic compounds are utilized in pharmaceutical, cosmetic, and food industry [15,16].

Plants have played a key role in human life for as long as they have existed. A wide variety of secondary metabolites, endowed with potential biological activities, are synthesized from plants. The genus *Quercus* includes more than 500

species growing in temperate ecosystems [17]. Oak acorns, one of the species of *Quercus* genus, are of vital importance for both humans and animals. They have been widely used as food for many thousands of years in many regions worldwide [18]. According to Bainbridge [19] acorns were a staple food throughout Europe, the Mid-East, North Africa, Asia, and North America.

Acorns are nutritional dense functional food with health properties. Some of the health benefits are attributed to the high level of phenolic compounds found in acorns. These phenolic compounds provide acorn fruit with high levels of antioxidants, which could have potential health benefits [18,20].

The aim of the present study was to compare total phenolic and flavonoid contents, as well as the antioxidant activity of both flour and oil extracts of two acorn species grown in Algeria: *Quercus ilex* L. (*Q. ilex* L.) and *Quercus suber* L. (*Q. suber* L.).

2 Material and methods

2.1 Plant collection

Acorn fruits were directly gathered from two or three individual trees from Oum El Bouaghi region (Sidi Rghis mountain) east of Algeria. *Quercus* species, used in this study, were identified by comparison with those already published in the "Illustrated Guide to Algerian Flora" (N° 978-2-7466-4242-3). Collected seeds (acorns) were sorted and cleared of all impurities, separated from the shell then finely powdered using electric grinder and passed through a 425 µm sieve. The obtained powder was freeze-dried at -50°C and put under pressure of 0.08 mbar for 24 h to obtain a moisture content <1% to undergo extraction.

2.2 Oil extraction

Acorn oils were extracted using Soxhlet method. Acorn powder (10 g) was weighed into a cellulose extraction cartridge and the Soxhlet apparatus containing the cartridge was fitted to a distillation flask containing 100 mL n-hexane. After 6 hours of extraction, the extract was filtered and dehydrated with anhydrous sodium sulphate and the solvent was evaporated under vacuum at 50°C. The amount of oil in the fruits was expressed on a dry-weight basis [21].

2.3 Physical and chemical constants

AOCS methods [22] were used to determine free acidity, peroxide value, iodine value and ultraviolet absorption indices (K_{232} , K_{270} , and ΔK).

2.4 Extraction of phenolic compounds

The prepared samples (oils and flours) were subjected to an extraction of their phenolic compounds. The extraction of phenols from the *Quercus* oils was carried out according to the procedure described in Squeo *et al.* [23,24]. To obtain the extract, 1 g of oil was dissolved in 1 mL of hexane and 5 mL of methanol/water (70:30 v/v). The mixture was vortexed during 10 min and centrifuging at 3941 g for 10 min at 4 °C (Beckman Coulter, Fullerton, California, USA). The methanolic phase was recovered, centrifuged again at 8867 g for 5 min at 4°C, and finally filtered through 0.45 mm pores filters. The extracts were analyzed for determination of total phenols and for flavonoid contents.

Polyphenol extraction from the *Quercus* flour was performed as reported by Difonzo *et al.* [25] with some modifications. Approximately, 5 g of homogenized sample was added to 50 mL of methanol/water (70:30 v/v). The mixture was subjected for 35 min to ultrasound (CEIA, Vicinaggio, Italy) treatment at room temperature (energy intensity 1.96 kWh L⁻¹). The obtained extracts were filtered through Whatman filter paper (GE Healthcare, Milan, Italy), then filtered with nylon filters of 0.45 µm (Sigma Aldrich) and used for chemical characterization. For each species, two different extraction solvents were utilized.

The extracts were analyzed both for determination of total phenols and flavonoid contents.

2.5 Extracts characterization

Total phenols

The total phenolic content was quantified by the Folin-Ciocalteu method using a calibration curve of gallic acid ($R^2=0.9979$), according to Singleton & Rossi [26] with the modifications reported by Caponio *et al.* [27]. Briefly, 100µL of appropriately diluted extract were mixed with 100 µL of Folin-Ciocalteu reagent. After 4 min, 800 µL of Na₂CO₃ solution 5% (w/v) was added to the mixture and heated in a water bath at 40 °C during 20 min. After being cooled down for 15 min, the absorbance of the solution was measured at 750 nm by an Agilent Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, USA). The total phenolic content was expressed as gallic acid mg equivalents kg⁻¹ of oil and mg 100 g⁻¹ dry matter for the flower.

Flavonoids

Total flavonoid content was quantified using a spectrophotometer according to Cosmai *et al.* [28]. Briefly, 500 µL of each extract was mixed with 2 mL of distilled water and 150 µL of NaNO₂ solution (5 %). After 6 min, 150µL of a 10% solution of AlCl₃ was added and allowed to stand further 6 min; thereafter, NaOH solution (2 mL, 1 M) was added to the mixture. Then, distilled water was added to bring

the final volume to 5 mL. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was read at 510 nm. The results were expressed as mg of catechin equivalent per kg of oil mg 100 g⁻¹ dry matter for the flour by means of a catechin standard which was made in the same conditions ($R^2=0.999$).

2.6 Antioxidant activity

ABTS assay

The antioxidant activity was evaluated on the basis of the scavenging capacity of the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt) compared with a reference antioxidant standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as described in Difonzo *et al.* [25]. Fifty microliters of each sample were added to 950 μ L of ABTS reagent previously prepared. After 8 min, the decrease of absorbance was measured at 734 nm. Calibration curve of Trolox was used, and the results were expressed in μ mol Trolox equivalents (TE) g⁻¹ of sample (oil and flour).

DPPH assay

Extracts were analyzed for their capacity to scavenge the stable DPPH radical (1,1-diphenyl 2-picrylhydrazyl free radical) as a free radical using the method of Brand-Williams *et al.* [29]

cuvettes for spectrophotometry, 50 μ L of each sample were added to 950 μ L of DPPH solution (0.08 mM in ethanol). After 30 min, in the dark, the decrease of absorbance was read at 517 nm. The results were expressed in μ mol TE g⁻¹ of sample.

2.7 Statistical analysis

All analyses were carried-out in triplicate and the experimental data were reported as means \pm standard deviation. Analysis of variance (one-way and two-way ANOVA), Tukey's test and correlation analysis were performed on the experimental data using XLSTAT software.

3 Results

3.1 Oil contents and quality indices

The oil contents of acorn ranged from 7.05 % for *Q. ilex* L. to 7.83 % for *Q. suber* L. species [30]. The physical and chemical constants, estimated in the oil extracted from the two Quercus species, are shown on Figure 1. As revealed, the acidity value was significantly low in the two oils and especially in *Q. ilex* (0.97 g/100g). Acorn oils showed also the lowest peroxide value which remained < 1.5 meq O₂ kg⁻¹ oil. *Q. ilex* L. oil showed slightly higher value of PV, while *Q. suber* L. was characterized by the highest acidity values. While, no significant differences were found in ANOVA ($p < 0.05$) between the two species.

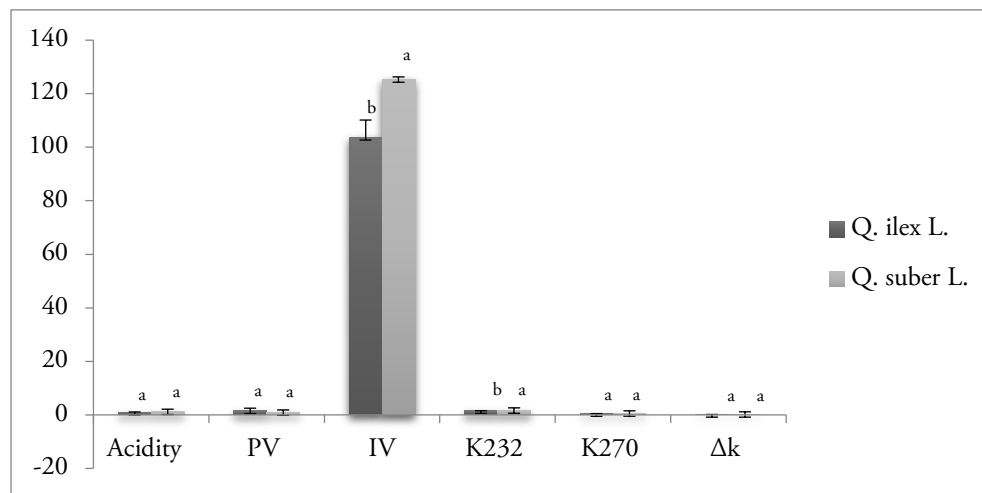


Figure 1. Physical and chemical characteristics of Quercus fruit oils

PV, peroxide value; K₂₃₂, specific extinction at 232 nm; K₂₇₀, specific extinction at 270 nm; IV, iodine value. Values are expressed as mean of three replications \pm S.D. Different letters indicate significant differences at $p \leq 0.05$ according to one-way ANOVA followed by Tukey's HSD test

following a modification of the procedure described by Difonzo *et al.* [25]. This assay is based on the abilities of the antioxidants present into the extracts to scavenge the radical in comparison with that of the standard antioxidant (Trolox). In

Similar to PV and acidity values, slight amounts of all spectrophotometric parameters measured (K₂₃₂, K₂₇₀, and Δk) were found. As shown on Figure 1, relatively high iodine value

of the two oil samples was observed and *Q. suber* L. had the highest value.

Water furnished an extraction yield of $14.87 \pm 4.42\%$ which remained weaker than the mixture water/methanol (50:50), methanol and ethanol solvents. Moreover, the evaporation of water took more time and required more energy consumption, which reduces its use. For that reason, some authors advice against using water [15] for phenol extraction, despite its safety and low price.

3.2 Phenolic content

As shown on Figure 2, total phenolic contents (TPC) are given as gallic acid equivalents by reference to standard curve. Total phenolic contents of Quercus fruits showed highly significant differences ($p \leq 0.05$) depending on species and extracts. Quercus flour possessed the highest level of total phenolic contents (1101–1464 mg GAE/kg dry weight), while the lowest content was recorded for the extracts obtained from Quercus oil (195.6–322.06 mg GAE/kg oil). The highest level of TPC was observed in *Q. suber* L. for the two extracts.

3.3 Flavonoid content

The results obtained after determining flavonoids, throughout the aluminum chloride method, are illustrated on Figure 2. Flavonoid contents (TFC) showed significant differences too ($p \leq 0.05$) and depend on varieties and extracts. As can be observed on Figure 2, flavonoid contents were ranged between 212.26 and 279.82 mg CE/kg dry weight and 122.99 and 131.6 mg CE/kg of oil for Quercus flour and Quercus oil, respectively. The significant higher amount was obtained in *Q. ilex* L. species for the two different extracts.

3.4 Antioxidant capacity

The antioxidant activity of different extracts was assessed using DPPH•+ and ABTS•+ radicals, and the results are summarized on Figure 3. The antioxidant activity's analysis reflects a high significant variation ($p \leq 0.05$) between the two extracts. As presented on Figure 3, flour extract of *Q. ilex* L. species exhibited the strongest scavenging capacity on DPPH (52.62 g TE/g dry weight). ABTS scavenging activity is also one of the most commonly used method to evaluate the antioxidant properties in fruits and plants. This activity varied from 1.27 to 3.23 mg TE/g for acorn oil and from 36.19 to 44.50 mg TE/g for acorn flour.

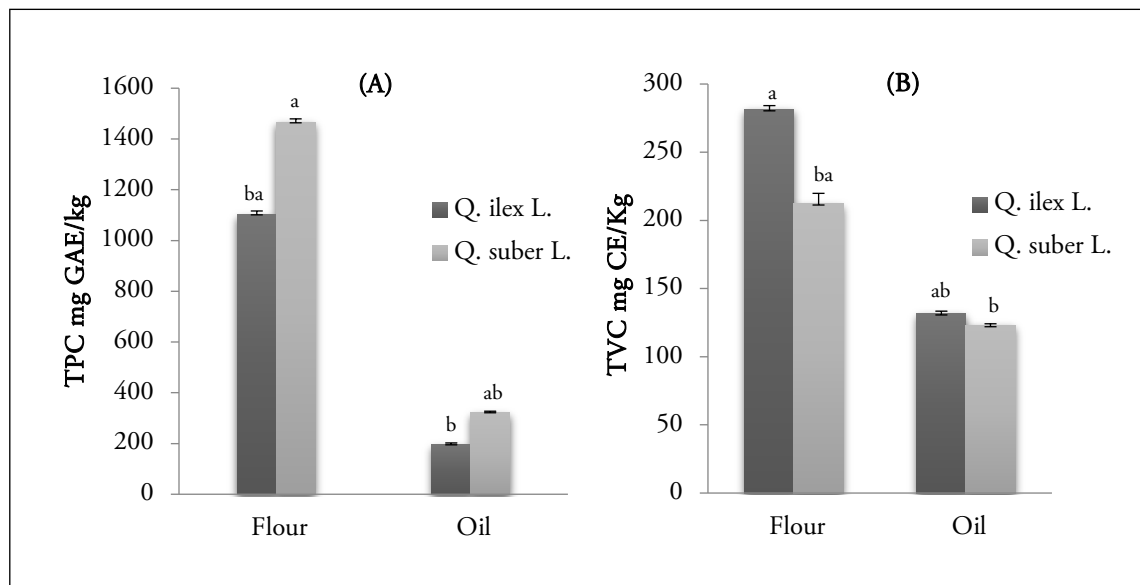


Figure 2. Total phenolic content (A) and total flavonoid content (B) of Quercus fruit oil and flour
 TPC, total phenolic content; TFC, total flavonoid content. Values are expressed as mean of three replications \pm S.D. Different letters indicate significant differences at $p \leq 0.05$ according to one-way ANOVA followed by Tukey's HSD test.

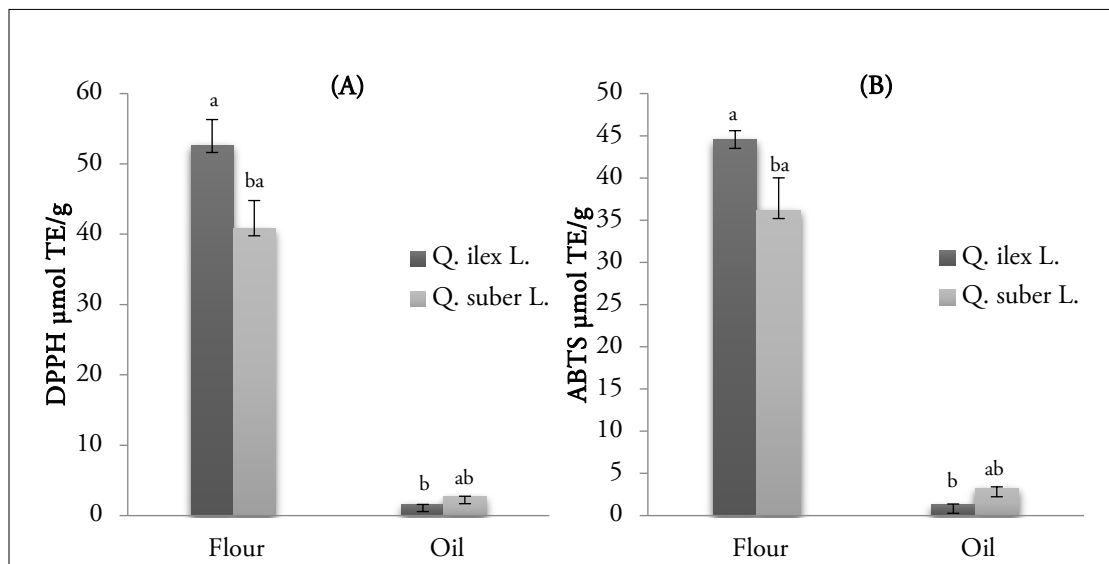


Figure 3. Antioxidant activity of the phenolic fraction ($\mu\text{mol TE g}^{-1}$) of *Quercus* fruit oil and flour: (A), DPPH test; (B), ABTS test

Values are expressed as mean of three replications \pm S.D.

Different letters indicate significant differences at $p \leq 0.05$ according to one-way ANOVA followed by Tukey's HSD test

4 Results

The oil contents, obtained from *Quercus* species, agree with previous findings that reported a range of 6-9% from some acorn species [31,32]. This demonstrated that those contents are low for commercial production as cooking or frying oils and the acorn fruit cannot be considered as oleaginous seed that possess 30-45% of oil [33]. However, there is a possibility of using this kind of oil as a supplement ingredient in products. In addition, acorn oil might be considered similar to other plant oil sources used because of their health benefits or their industrial and pharmaceutical applications [32,34].

Peroxide value, free acidity, and ultraviolet absorption are considered to be fats and oils indicators of quality and stability [35]. The main quality characteristics used were found within the ranges for vegetable oils of good quality [36]. The relatively low acidity values indicate that the *Quercus* oils contain a slight amount of free fatty acids. The low peroxide value indicates an absence of oxidation, which is confirmed by the low presence of primary (K_{232}) and secondary oxidation products (K_{270}) [37].

The iodine value is a measure of the total number of double bonds present in fats and oils [37]. It provides an overall status of unsaturation [38]. High iodine-value oil contains a greater number of double bonds than low iodine-value one [39]. Therefore, the relatively high iodine value of the two oil samples may be indicative of the presence of many unsaturated bonds and would certainly contain more unsaturated fatty acids [30].

The amount of phenolic compounds constitutes an important determination since natural phenols improve its resistance to oxidation. These compounds have been correlated with the shelf life of oil, in particular, regarding to its antioxidant activities [40-42].

A comparative study has been carried out between two different extracts (oil and flour) of *Quercus* fruit. As shown previously, the total phenolic and flavonoid contents were detected at levels significantly higher in the two species of acorn flour. Our results showed higher TPC than that already reported in acorn fruits from other countries of Mediterranean basin [43,44].

On the contrary, the content of phenolic compounds was significantly lower in acorn oil extracts, while the flavonoids did not show any large significant differences. The low amount of phenolic compounds in acorn oil was probably due to the loss of part of these components during oil extraction.

The flavonoids' amount was remarkable in acorn oils and flour respect to other vegetable oils and seeds. For instance, in virgin olive oils, the flavonoid content is generally low, ranging from few mg kg^{-1} to around 50 mg kg^{-1} [45-47].

In addition, noticeable differences of total phenolic and flavonoid contents were observed among cultivars *Q. ilex* L. and *Q. suber* L. in both extracts (oil and flour). These minor variations observed might be related to the difference in acorn species, environmental conditions, and oak acorn maturity.

Antioxidant activity analysis reflected as well a wide significant variation between the two *Quercus* extracts. *Quercus* flour extract exhibited the strongest scavenging capacity against DPPH• and ABTS•+ radicals. These findings were directly related to the higher content of *Quercus* flour in scavenging agents of free radicals acting as antioxidants. Cantos *et al.* [48] have obtained similar results by measuring the antioxidant activity of the phenolic fraction of three *Quercus* species by these two radicals.

The significant antioxidant activity of *Quercus* fruit is due to the inductive effect of the natural antioxidants present in the fruit such as phenolic compounds and flavonoids which reduce and discolor free radicals (DPPH•, ABTS•+) because of their ability to yield hydrogen [49,50].

Correlation analysis was used to establish the relationship between antioxidant activity and the different variables measured (Table 1). A strong significant correlation ($p < 0.001$) was observed between scavenger activity on free radicals (DPPH•+, ABTS•+) and total phenolic content. Flavonoid compounds showed in turn a high correlation ($p < 0.001$) between their content and the antioxidant properties.

Table 1: Linear correlation between phenolic compounds and antioxidant activities

Variables	Pearson correlation coefficient r	P
TPC _{oil} -DPPH	0.983	***
TPC _{oil} -ABTS	0.915	***
TPC _{flour} -DPPH	0.821	**
TPC _{flour} -ABTS	0.756	**
TFC _{oil} -DPPH	0.836	***
TFC _{oil} -ABTS	0.925	***
TFC _{flour} -DPPH	0.746	**
TFC _{flour} -ABTS	0.649	**

** $p < 0.01$: significant correlation; *** $p < 0.001$: high significant correlation. TPC, total phenolic content; TFC, total flavonoids content.

5 Conclusion

The present study brings new insight of two Algerian acorn species, particularly their phenolic, flavonoid contents, and their antioxidant activity. Antioxidant properties, total phenolic, and flavonoid contents differed significantly within the two studied extracts. Flour extracts showed strong antioxidant properties and high total phenolic content. Based on the correlation analysis, a positive relationship between antioxidant capacity and total phenolic contents was found, indicating that those phenolic compounds constitute the major contributors to the antioxidant properties of these plants. Our results showed that the *Quercus* fruit (oil and

flour) represents an interesting natural antioxidant source containing various biological active compounds. Therefore, it is suggested that the *Quercus* fruit could be used as a functional ingredient in food industry allowing an efficient protection against oxidative deterioration, and as a valuable phytochemical source for preventing human diseases in which free radicals are involved, such as diabetes and cardiovascular diseases.

Conflicts of interest: The authors declare no conflicts of interest.

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