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# Biological and phytochemical characterization of takermoust date syrup

**Ymina Mimouni*, Zahia Bayoussef, Oumelkheir Siboukeur**

*Laboratory of Water and Environmental Engineering in Saharan Mid. University Kasdi Merbah-Ouargla, Faculty of Natural and Life Sciences, Department of Biological Sciences. Ghardaïa road 30 000 Ouargla, Algeria*

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## Abstract

The cultivation of the date palm is, until today, a main source of life for the populations of the Saharan regions. It represents both the basis of agricultural activity and a source of food. The present study aims to develop date syrups based on the low-value cultivar Takermoust (T) and the cultivar Ghars (G) (control), as part of contributing to the enhancement of this phoenicultural heritage and the innovation of a dietetic product by a technological method. The method adopted is based on the diffusion of soluble solids in water at two concentrations, temperatures 65 °C and 105 °C. Qualitative characterization was targeted by phytochemical screening and thin-layer chromatography (TLC). Quantitative analysis was performed by assaying phenolic compounds and evaluating biological activity. TLC reveals the presence of rutin-like flavonoids and gall tannins. The polyphenol content varies between 0.82 - 0.94 mg gallic acid equivalent /100g of syrup, the flavonoid content fluctuates between 0.38-0.64 mg equivalents of rutin/100g of syrup. T65 ° C syrup shows significant antioxidant activity (165 mg equivalent of ascorbic acid/100g of syrup). The evolution of the anti-free radical activity seems interesting for all the syrups; the T 65 °C syrup evokes a significant inhibitory concentration of 0.028 mg/ml with a percentage of inhibition of 68.81%. The concentration at 65 °C seems better for the preparation of dietetic syrups. Overall, the syrups made from Takermoust dates show an interesting phytochemical composition and biological activity against the control cultivar Ghars. So, they can integrate these therapeutic virtues into the diet of the local population.

* **Corresponding Author:** Ymina Mimouni ✉ yamina.mimouni@yahoo.fr

## Introduction

The date palm (*Phoenix dactylifera* L.) is the most important crop in arid and semi-arid areas. It plays an important role in the economic and social life of the populations of these regions. It is considered the tree of the desert regions of the world known for its hot and dry climate. Due to its nutritional, ecological, social and economic virtues, the date palm is the fruit tree most appreciated by the populations of the oases.The fruits of the date palm (dates) are widely consumed by the population of the region. They are very numerous, only a few are of commercial importance and others are of low market value. Dates are differentiated by flavor, consistency, shape, color, weight and dimensions(Djerbi, 1994; Gheraissa and Hamidani, 2018).They constitute a very important energy source (more than 60% of sugar), in addition to other essential nutrients for the human body such as amino acids, antioxidants and some mineral elements (Chafi *et al.*, 2015).Dates are generally rich in fiber, facilitate intestinal transit and play a preventive role in colorectal cancer, appendicitis, diverticulosis, varicose veins and hemorrhoids. They also have a cholesterol-lowering effect (Ben abbas, 2011). These fruits help fight anemia and demineralization, so they are recommended for breastfeeding women. Algeria has no processing technology, except for the packaging and production of "Ghars" paste from soft dates. Many date palm cultivars remain poorly exploited or even marginalized.In order to contribute to the safeguard of the phoenicultural heritage (more than 1000 cultivars) threatened by genetic erosion, it is important to find serious outlets for this fruit (common dates) through development trials of new products, including a date syrup by technological means.Date syrup is highly recommended (children, convalescents, pregnant women). It confers important properties in terms of nutrient supply.In this context, we proposed to develop date-based syrups by a technological method from a cultivar of low commercial value (Takemost) and a cultivar of significant commercial value (Ghars) as a control, in the framework of contributing to the enhancement of this phoenicultural heritage on the one hand and innovation of a dietetic product on the other hand by the characterization of its phytochemicals transferred by its raw material in which they are derived.

## Material and methods

### *Material*

The material used in this study is based on plant material (date cultivars). The choice of the cultivar Takermoust is justified by their relative abundance in the region of South-East Algeria (palm grove of El Kser in Ouargla), their low market value, their taste quality, their classification among cultivars subject to genetic erosion and their nutritional value. The dates of the cultivar Ghars were chosen as a control. They were harvested from a palm grove in Hassi Ben Abdellah, Ouargla. The dates of the two cultivars studied were harvested starting September 2020 at the stage of complete maturation (soft consistency) (Fig.1). In general, these cultivars can be eaten as they are or intended for technological processing into several products, including date syrup.

### Methods

*Preparation of syrups*

To have a good quality product, it is important to start with a good quality raw material. The extraction method adopted in this present study is based on simple diffusion laws. This method allows the passage by passive transport of soluble solids from the plant cells of the dates to the solution (hot water or juice) through the cellulose membrane(Alberts *et al.*, 2002).It is done hot, at 80 ° C, by adding one weight of dates to three volumes of distilled water (w / v). The juice is concentrated by evaporation of free water at 65 ° C (low temperature) and 105 ° C (high temperature). The purpose of evaporation is to obtain a saturated syrup with a Brix degree between 72 - 74 ° Brix.

*Qualitative analysis of date syrups*

Qualitative analysis shows the presence of a few chemical compounds transferred from dates. The analysis is carried out by phytochemical tests of color reactions (phytochemical screening) and thin-layer chromatography (TLC). The phytochemical groups

testedare alkaloids, polyphenols, flavonoids, anthocyanins, tannins, saponins, steroids, coumarins, sterols, terpenes, cardiotonicheterosides, essential oils, reducing sugars. The principle of these tests is based either on the formation of insoluble complexes using precipitation reactions or on the formation of colored complexes using coloring reactions. TLC is performed on silica gel plates (60 F254, aluminum support, 20 × 20, Merck). The mobile phase consists of the solvent system butanol, acetic acid, distilled water (12/3/5). Three revealing preparations were used, namely: Aluminum chloride (5%) in Ethanol, UV lamp at 254 nm and a solution of iron chloride (10%) dissolved in ethanol.

*Evaluation of biological activity*

The determination of the phenolic compounds is carried out in the presence of Folin-Ciocalteu reagent. This reagent contains a mixture of complexes of phosphotungsten and phosphomolybdenum acids of yellow color. The principle of this method is based on the oxidation of phenolic compounds by this reagent; it leads to the formation of a new blue molybdenum-tungsten complex which absorbs at 760 nm, measured with a spectrophotometer (UV-visible model DR 5000 HACH LANGE).The concentration of phenolic compounds is calculated from a calibration curve of gallic acid and expressed in milligrams of gallic acid equivalent /100g of date syrup. The flavonoids assay is based on the formation of a yellow complex between the aluminum chloride and the oxygen atoms present on the 4 and 5 carbons of the flavonoids; the absorbance is measured at 410 nm. The concentration of flavonoids is determined using a rutin calibration curve. The results are expressed in milligrams of rutin equivalent/100g of date syrup (Hassan *etal*., 2010).This analysis was supported by ANOVA (analysis of variance) statistical analysis by Tukey's model.

*Antioxidant activity*

The antioxidant activity of date syrups is tested by the phosphomolybdate test and the 1,1-Diphenyl-2-picryhydrazyl (DPPH) free radical scavenging assay. The phosphomolybdic acid test evaluates the total antioxidant activity. The test is based on the reduction of phosphate-Mo (VI) to phosphate-Mo (V) by antioxidants and the formation of a bluish-green phosphate Mo (V) complex at acidic pH (Phatak and Hendre, 2014). Absorbance is measured at 695 nm. Ascorbic acid is used as a standard and the results are expressed in milligrams of ascorbic acid equivalent per 100g of date syrup.

The anti-free radical activity was evaluated by measuring the scavenging power of the DPPH radical (1,1-Diphenyl-2-picryhydrazyl). The test is based on the reduction of the violet radical (picryhydrazyl) by antioxidants, to hydrazine (picryhydrazine) of pale yellow color. The change in color indicates the anti-free radical activity (the free radical scavenging activity of the sample); the absorbance is read at 517 nm. The Inhibitory Concentration ($IC_{50}$) is defined as the concentration of antioxidants required to decrease the initial concentration by 50%; it is related to the antioxidant capacity. Therefore, the lower the antioxidant capacity, the greater the $IC_{50}$.

**Results and discussion**

*Properties of date syrups*

The Brix degree recorded after the concentration of the filtrates of two cultivars at 65 °C and 105 °C are summarized in Table 1. Overall, these values vary between 72 - 74 °Brix. These results can be explained by the fact that the extraction temperature of 105°C allows a greater amount of (free) water to be removed in a shorter time. These values are closer to the range cited by Mimouni (2015) (72 - 73 °Brix).

**Table 1.** Rate of soluble solutions (°Brix) and yield (%) of date syrups obtained at two temperatures

| Date syrups | °Brix | Yield |
|---|---|---|
| G 65°C | 72 ± 2.12 | 29 ± 0.13 |
| G105°C | 73 ± 1.20 | 31.2 ± 2.18 |
| T65°C | 72 ± 0.5 | 25.8 ± 1.23 |
| T105°C | 74 ± 1.40 | 27.8 ± 1.90 |

A double extraction of date syrups at two concentration temperatures, that is to say,concentration by direct heating (105 °C) and by

evaporation (65 °C), gives a relatively interesting yield for G105 °C (31.2%) compared to syrup G 65 ° C (29%), T105 ° C (27.8%) and T 65 °C (25.8%) (Table 1). This is probably due to the richness of dates in soluble solids and/or the high extraction temperature (extensive extraction at high temperature). Date syrup extraction yields are directly related to the extraction method used.

The extraction yield of date syrup by traditional methods (tamping) varies between 10 to 15% (Abdelfattah, 1990; Siboukeur, 1997).

**Table 2**. Photochemical screening of date syrups of cultivars Ghars and Takermoust.

| | Ghars syrups | | Takermoust syrups | |
|---|---|---|---|---|
| Secondary metabolite | G 65°C | G 105°C | T 65°C | T 105°C |
| Flavonoids | + | + | + | + |
| Tannins (catechetical) | + | + | + | + |
| Coumarins | + | + | + | + |
| Anthocyanins | - | - | - | - |
| Alkaloids | - | - | - | - |
| Terpenoids | + | + | + | + |
| Saponosides | - | - | - | - |
| Cardiotonic glycoside | + | + | + | + |
| Steroids | - | - | - | - |
| Sterols | - | - | - | - |
| Essential oils | - | - | - | - |

The color of the syrup depends on the extraction treatment used, the color of the date and the storage time. The date syrups that are the subject of this study are obtained by the diffusion extraction method. They exhibit a dark amber color at two temperatures (65 °C and 105 °C). The extraction temperature did not seem to affect the color of the syrups.

These syrups are clear, which eliminates the need for clarification processes (Fig. 2. A, B). Concentration or dehydration is a physical property of water in food. Technological treatments such as heating modifications of the environment cause the water content of food to vary (Alais and Linden, 1987). However, the biological activity of water (aw) is essential in food since it makes it possible to implement a food protection strategy by controlling physicochemical deterioration, enzymatic activities and the growth of microbial populations (Alais and Linden, 1987).Concentration remains an essential operation to reduce the development of microorganisms, inhibit enzymatic reactions and facilitate storage and, therefore, the preservation of the product (low aw) (Cheftel and Cheftel, 1984).

**Table 3.** Variation in the content of phenolic compounds and flavonoids in date syrups depending on the ltivars.

| Sample / Concentration temperature (° C) | | a *phenolic compounds | b* flavonoid |
|---|---|---|---|
| 65 | Ghars | 0,94 (a) | 0,610 (a) |
| | Takermoust | 0,94 (a) | 0,397 (b) |
| 105 | Ghars | 0,86 (a) | 0,640(a) |
| | Takermoust | 0,80 (a) | 0,380 (b) |

[a*]mg GA eq /100g date syrup , [b*]mg eq rutin /100g of date syrup, Mean ± SEM, [a] P ≤ 0.05.

The results of the phytochemical tests are recorded in Table 2. From this table, we note the presence of flavonoids, tannins, coumarins, terpenoids, cardiotonic glycosides, sterols and terpenes. The absence of anthocyanins, alkaloids, saponosides, steroids and essential oils was absent in the syrups of both cultivars.

**Table 4.** Antioxidant activity of date syrups estimated mg equivalent of ascorbic acid /100 of date syrup studied.

| date syrup | Antioxidant activitymg |
|---|---|
| G 65°C | 130 ± 22.16 |
| G105°C | 115 ± 18.03 |
| T65°C | 165 ± 30.13 |
| T105°C | 150 ± 24.40 |

The phytochemical screening of the secondary metabolites sought, shows that the results of all the date syrups produced at two concentration temperatures (65 ° C and 105 ° C) are comparable, this leads us to say that the concentration temperature did not influence the phytochemical quality of date syrups. After the revelation by aluminum trichloride followed by revelation under a UV lump of the TLC plates, we note the presence of brown, gray spots (Frontal ratio: Rf) represent gallic acid (0.88), catechin (0.65) and rutin (0.83) with a yellow spot. Four (Cheftel and Cheftel, 1984) spots of yellow color, Brown evoke the syrups of the cultivars Ghars G65 ° C (0.52), G105 ° C (0.56) and Takermoust T65 ° C (0.55), T105 ° C (0.55) (Fig. 3).The Rf of date syrups of the cultivars studied seem comparable; the yellow color characterizes the presence of flavonoids.Aluminum trichloride was used to reveal flavonoids by the appearance of the yellow color (Khan *et al.*, 2011). Likewise, (Sayah, 2018) showed the presence of yellow spots for the cultivar Degla-Beida (Rf: 0.46), the cultivar Deglet-Nour (Rf: 0.44) and the cultivar Ghars (Rf: 0.41) after the revelation by aluminum trichloride.

*Quantitative characterization*

The polyphenol content of date syrups of the cultivars studied is presented in Table 3. The values obtained for the syrups of two cultivars seem comparable for the same concentration temperature: G 65 ° C (0.94), G105 ° C (0,86), T65 ° C (0,94) and T105 ° C (0.80)in mg equivalent of gallic acid/100g of date syrup, however, these values are slightly elevated at the temperature 65 ° C, this may be due to the effect of the high concentration temperature, this one probably affects the phenolic composition (modification).According toBen Abbas,(2011),the polyphenol content for crude extracts of Ghars, Deglet-Nour and Degla-Beida varies between 2.91 and 5.89 mg gallic acid equivalent / 100g of dates. The values recorded during the present study are low compared to those cited; this is probably due to the extraction temperature, which could affect the content of polyphenols.

**Table 5.** Inhibitory concentration of the date syrups.

| Date Syrups | IC50(mg/ml) |
|---|---|
| G 65°C | 0.0385 |
| G105°C | / |
| T65°C | 0.0285 |
| T105°C | 0.0280 |

In this study, it is noted that the low concentration temperature (65 °C.) appears to be better for extracting the polyphenols. Previous studies had discussed the influence of different factors on polyphenol content such namely, the stage of ripening of dates, the date cultivar, maturity, storage conditions, use of fertilizers, type of soil, season, the geographical origin and the quantity of light received (Al-farsi *et al.*, 2008). Table 3 also shows the flavonoid contents obtained for the date syrups of two cultivars studied: G 65 ° C (0.61), G105 ° C (0.64), T65 ° C (0.38) and T105 ° C (0.39) in mg equivalent of rutin/100g of date syrup. Tukey's test shows a significant difference between the two cultivars and at two temperatures.

The values obtained with Ghars syrups are higher than those recorded for syrups of Takermoust. Differences between date cultivars in flavonoids had been evident even in the fresh form. A study by Ben Abbas, (2011) suggests that the content of flavonoids

in fresh matter fluctuates between 0.33 and 0.66 mg equivalent of rutin / 100g. Overall, the flavonoid content of dates cited in the literature or that mentioned in the present study remains significant. The values presented in table 3 even suggest that the preparation of syrups itself does not have a negative impact on flavonoids irrespective of concentration temperature.


**Fig. 1.** Cultivars, A, Ghars ; B, Takermoust.

*Antioxidant activity*

Antioxidant activity is expressed by the ability of antioxidants to scavenge free radicals found in biological systems and to end the chain reaction before vital molecules are damaged **(**Kchaou *et al.*, 2013;Khan *et al.*, 2011).

It is expressed in milligrams of ascorbic acid equivalent per 100g of date syrup. Table 4 represents the results of the antioxidant activity of date syrups of two cultivars studied, namely: 130, 115, 165 and 150 mg equivalent of ascorbic acid / 100 g of date syrup for G65 ° C, G105 ° C, T65 ° C and T105 ° C respectively. Date syrup from cultivar T65 ° C shows significant activity compared to the other three samples studied; these results can be explained by its richness in other unidentified phenolic compounds than polyphenols and flavonoids. The concentration temperature at 65 ° C seems honeyed in a way to help preserve the activity of these syrups.

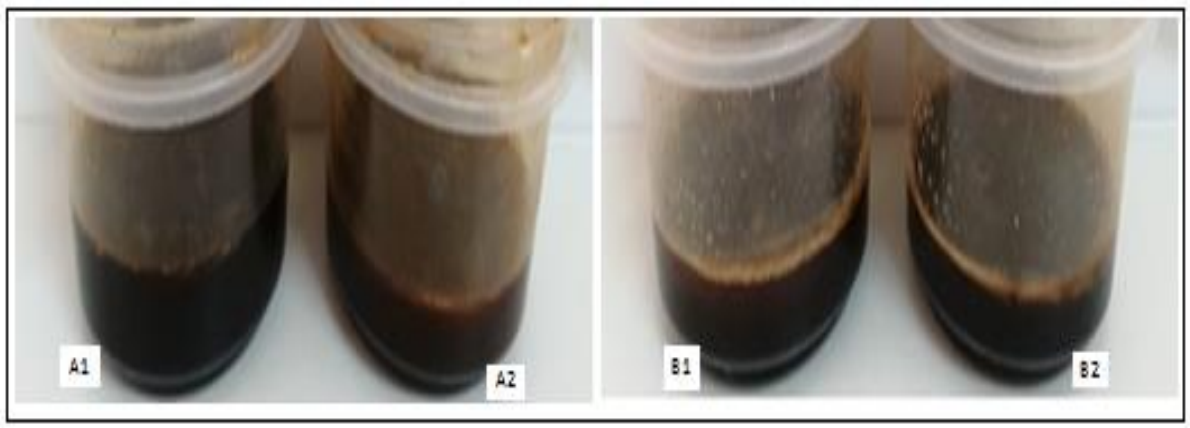


**Fig. 2.** Cultivars date syrup; (A) Ghars, A1: G65°C, A2: G105°C ; (B) Takermoust, B1 :T65°C, B2 ; T105°C.

The results mentioned by Sayah (2018) recorded a total antioxidant activity by ethyl acetate extract of the cultivar Ghars at the Routab stage with 38.75 mg equivalent of ascorbic acid / 100g of dates, followed by the extract of n-butanol from the immature part of dates of the same cultivar at the Routab stage with 23.73 mg equivalent of ascorbic acid / 100g of dates. These values seem low compared to those mentioned in the present study. The process of concentration of syrups which increases the content in soluble solids

(which include polyphenols) appears to privilege a high antioxidant activity.According to Kchaou *etal.* (2013), the total antioxidant activity of six cultivars of dates from Tunisia is between 17.49 ± 3.19 and 109.67 ± 2.04 mg equivalent of ascorbic acid /100 g of fresh weight. The antioxidant activity of dates and their products could provide phytochemicals including polyphenols, flavonoids, tannins, carotenoids, anticyans, etc (Al-farsi *et al.*, 2005). It is also important to note that some sugars present in dates are endowed with antioxidant properties (Gourchala, 2015).

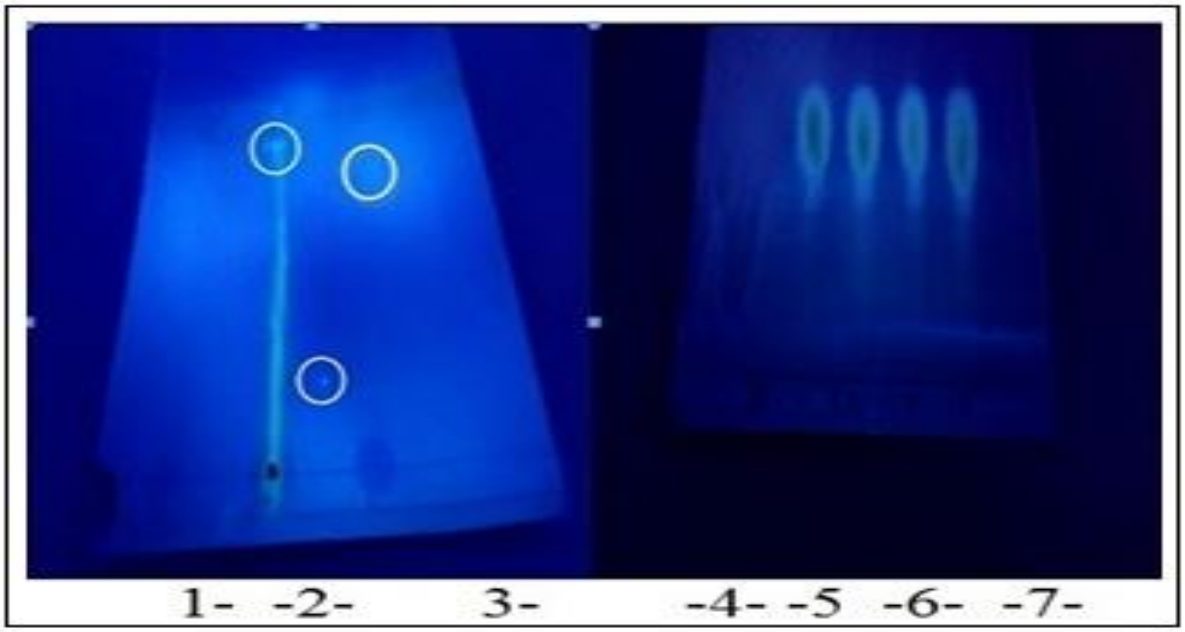


**Fig. 3.** Thin-layer chromatography of date syrups (revealing with 5% aluminum chloride); 1: Galic acid, 2: Catechin 3: Ritin, 4: G ° C65, 5: G ° C105, 6: T65 ° C and 7: T ° C105.

*Evaluation of anti-free radical activity*

The anti-free radical properties are measured and demonstrated by the Effective Concentration (EC50), which corresponds to the 50% reduction in the Concentration of DPPHin the reaction medium(Phatak and Hendre, 2014 ; Guillouty, 2016).

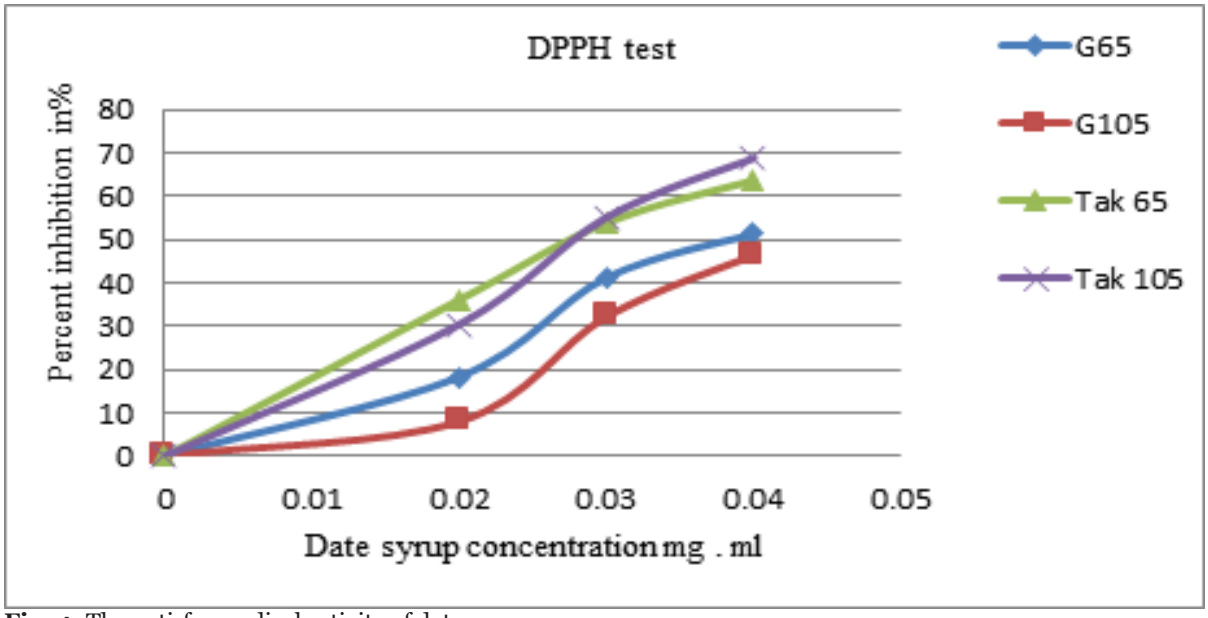


**Fig. 4.** The anti-free radical activity of date syrups.

The evolution of anti-free radical activity appears interesting for all the syrups of cultivars combined at different concentrations, except date syrup of Ghars cultivars 105 ° C (Fig. 4). The percentage of inhibition recorded is equal to 58.50%, 46.39%, 63.55% and 68.81% forG65 ° C, G105 ° C, T65 ° C and T105 ° C, respectively. The Takermoust cultivar date syrups at two temperatures exhibit an interesting anti-free radical activity given their higher percentage of inhibition, compared to other syrups.

The inhibitory concentration (IC50) is mentioned in Table 5. An exception is noted for G 105 ° C (IC50 indeterminable because of its low percentage inhibition). Sayah (2018) reports that the ethyl acetate extract of dates of the Ghars cultivar at the Routab stage (immature part) shows a great anti-free radical capacity with a percentage of inhibition of 74.21% at 10 mg/ml given its high content of polyphenols.

**Conclusion**

The phytochemical characterization of the syrups of the studied date cultivars of low market value Takermoust and the witness Ghars shows that the concentration at low temperature by evaporation appears to be better. The method adopted seems simple, low-cost, and can be used ona small scale. The content of phytochemicals (soluble solids) transferred from dates to syrups appears not to be negligible. Date syrups of all cultivars together exhibit an interesting phytochemical composition and biological activity. Overall, these elaborate syrups can incorporate these therapeutic properties into the diet of the local population.

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