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## *RESEARCH ARTICLE*

# QUALITATIVE DIAGNOSTIC OF CROCUS SATIVUS L. FROM DIFFERENT ENDEMIC ORIGINS OF TALIOUINE REGION IN THE SOUTH OF MOROCCO: HYGROSCOPIC BEHAVIOR AND MAIN SECONDARY METABOLITES ANALYSIS

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## *Abstract*

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This study examined the water activity and equilibrium moisture content of saffron stigmas from different aeras of Taliouine region, south of Morocco with a diversity of altitudes and climates. The static gravimetric method was used to explain the hygroscopic behavior of saffron at three different temperatures (30, 40, and 50 °C). The quality of these saffron samples was evaluated by determining the concentration of their main compounds: safranal, crocin and picrocrocin using the ISO 3632-2 method by UV-vis. The water activity for conservation of *Crocus sativus L.* was range between 0.32 and 0.34 under the experimental conditions. The impact of the three irrigation systems (drip, gravity and porous) on the water activity has been studied. All the six endemic origins identified by altitudes (2100 m, 1700 m, 1600 m, 1500 m, 1200 m and 1000 m) used in this diagnosis were classified according to ISO 3632-1 in two classes: categories I for saffron from altitudes 1700, 1600 and 1200 m and categories II for saffron from altitudes 2100, 1500 and 1000 m. The samples from altitude 1700 m recorded high quality of secondary metabolites represented by concentrations of crocin (291.68$\mathbf{E}^{1\%}_{440}$ ) and picrocrocin (120.11$\mathbf{E}^{1\%}_{257}$ ).



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## Introduction:-

The valorization of aromatic and medicinal plants has known in these last years - more particularly after the covid19- a remarkable evolution in several domains namely: agriculture, industry, cosmetic and pharmaceutical. As consumers of spices for example, we sometimes find ourselves lost in front of the different qualities of the same product; this variation can be due to several factors among them the method of conservation. Currently, drying is a critical issue for farmers and industrialists who seek to optimize the process in order to provide a good quality of the product respecting the standards of hygiene and food safety. Faced with this problem, we have studied in this work the technical solutions that describe the hygroscopic behaviour which precede the drying process. This step is characterized by determining the optimal conditions for saffron conservation, in particular the water activity which is a unique factor of food stability that allows the development of generalized limits in ranges where certain types of alteration reactions are dominant.

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Water activity is an effective concept commonly used in correlation with food safety and quality. Walter (1924) was probably the first researcher who was able to relate water vapor pressure to microbial growth, which is considered to be the main cause of food contamination (Jeantet, 2006)and the one who applied this relationship by introducing the concept of water activity ($a_w$) (Jeantet, 2006). One of the most useful approaches to the measurement of water availability is given by the constant $a_w$, hence the need for its control in food products including aromatic and medicinal plants. On the other hand, the sorption isotherm is a graphical representation that relates the equilibrium moisture content of a product and the water activity, at a constant temperature. The knowledge of sorption isotherms is very important in food science and technology, especially in the drying process, where they give us information about the optimal conditions for preservation and storage of a product.

Saffron (*Crocus sativus L.*) is a plant of the Iridaceae family, its dried stigmas are the part of saffron used as herbal medicine, culinary spice and for its coloring and taste characteristics. The Mediterranean environment is considered the most suitable area for producing saffron. L'Iran, l'Inde, la Grèce, le Maroc, l'Espagne et l'Italie sont les principaux pays où il est cultivé (Cardone et al., 2020). In Morocco, saffron is cultivated in a small southern region called Taliouine, located in the Anti-Atlas Mountains. The main areas of its cultivation were in altitudes of 1000 to 2100 m. The climate of the Taliouine region is characterized by a very hot season from June 12 to September 9, with an average daily maximum temperature of over 31°C. The hottest month of the year in Taliouine is July, with an average maximum temperature of 35 °C and a minimum of 21 °C. The cool season lasts from November 18 to March 6, with an average daily maximum temperature below 19 °C. The coldest month of the year in Taliouine is January, with an average minimum temperature of 3 °C and maximum of 16 °C.

The saffron's quality is assessed by its color, taste and aroma which are respectively due to the presence of three bioactive molecules: crocin, picrocrocin and safranal(Menghini et al., 2018). A higher amount of these components means that the saffron is of better quality. According to ISO 3632-2, safranal, crocin and picrocrocin are expressed as a direct reading of the absorbance of a 1% aqueous solution of dried saffron at 330, 440 and 257 nm respectively.

The choice of saffron in this research is justified by the fact that its stigmas fade and deteriorate rapidly after harvesting if they didn't immediately follow a good conservation process. Saffron from the Taliouine region is one of the products of the land that symbolizes the richness of the Moroccan heritage. Morocco is currently the fourth producer of this spice in the world.

In this work, we have chosen to study the hygroscopic behavior and the main bioactive molecules (safranal, crocin and picrocrocin) responsible of the metabolic activity of saffron. The sampling is carried out on six zones with different altitudes (2100, 1700, 1600, 1500, 1200 and 1000 m) in Taliouine region.

## Materials And Methods:-

### Sampling

The samples of the *Crocus sativus L.*(saffron stigmas) used in this study were taken in October-November 2019 from different areas of the Taliouine region. This is 3 communes (Assaiss, Tassoussfi and Taliouine) and six altitudes (2100, 1700, 1600, 1500, 1200 and 1000 m) distributed between the upper, middle and lower region of Taliouine (Figure 1).

Hygrothermal measurements and geographical coordinates of the sampling areas have been displayed in the table 1.The average rainfall in the region is shown in Figure2.

The flowers of *Crocus sativus L.*were collected early in the morning manually, which limits the length of their storage. These collected flowers are treated immediately to remove the stigmas, this is the pruning operation.

### Hygroscopic treatment

The relationship between the water activity of a product and its water content at a given temperature is called the sorption isotherm (adsorption or desorption). Sorption isotherms reflect the adsorption and water retention capacity of products. They have become major data in food technology (Machhour et al., 2012).

The determination of the sorption isotherms for the *Crocus sativus L.* plant was carried out by the static gravimetric method based on the use of six saturated salts at three temperatures 30, 40 and 50 °C in order to ensure that the water activity of the sampleremains variable within the range given in the literature which is between 5-90 %

(Mallek-Ayadi et al., 2020). Table 2gives the relative humidity values of selected saturated salt solutions as a function of temperature (Lahnine et al., 2016a).

Duplicate samples 0.02 ± 0.0001 g for desorption and 0.01 ± 0.0001 g for adsorption were weighed and placed in a sample holder which is placed in the glass jars. The glass jars are immersed in a thermostatically controlled bath to maintain a fixed temperature for 24 hours and bring the saturated saline solutions to a stable temperature. Indeed, mass transfers between the sample and the ambient air are guaranteed by the natural diffusion of water vapor (Lahnine et al., 2016a).

The mass of the samples is measured after every two days, until it becomes constant (equilibrium). Immediately afterwards, the samples are placed in an oven at 75 °C/24 h in order to determine their dry masses. Thus, the equilibrium water content of the samples is determined using the equation (1).

Table 3 regroups all equations used in this study, which it allows to determine the hygroscopic parameters related to moisture content. All these equations were created using "Math type" software.

Extrapolation of the sorption curves makes it possible to deduce the optimal water activity of *Crocus sativus L.* as well as its corresponding equilibrium water content.

**Sorption isotherms and modeling**

Numerous empirical and semi-empirical mathematical models based on physical principles that describe the relationship between equilibrium moisture content and water activity can be used to explain the experimental sorption data.

In order to characterize the hygroscopic properties of *Crocus sativus L.*, five mathematical isotherm equations were used (Azhar et al., 2021; Jamali et al., 2006).The sorption models used are listed and detailed in Table 4. These models were chosen for their applicability to food products and their physical significance. The non-linear regression analysis was performed using the CurveExpert Professional software. Each model had its own coefficients in each sorption temperature and evaluated by its correlation coefficient $R^2$ (Eq. (2)) and standard error SE (Eq. (3)).The best model that describes the hygroscopic behavior of *Crocus sativus L.* is the one with the largest $R^2$ and the smallest SE (Kane et al., 2008).

**Determination of optimal water activity**

Water activity directly determines the physical, mechanical, chemical and microbiological properties of many substances, such as: fluidity, coagulation, cohesion and static electricity. The ability to food preservation, color and taste stability, the content of vitamins, aroma and favorable conditions for the formation of mold and the growth of microbes are directly influenced by the value of water activity$a_w$. By measuring water activity in products, we can directly predict which micro-organisms represent a potential source of spoilage. It is water activity, therefore free water, which sets the lower limit of microbial growth. It has an important role in the design and optimization of drying equipment, food packaging, food quality, stability, and shelf life (Hamza et al., 2020a). For this, it is important to determine the optimal water activity of *Crocus sativus L.* The temperature, pH and some other factors have some influence on the possibility and rate of growth of an organism in foodstuffs. Water activity is the most important of these factors. Water activity is a critical factor that directly determines food preservation.

The study of adsorption-desorption isotherms makes it possible to know the optimum water activity of a product as well as its equilibrium moisture content. In addition, it provides researchers with precise information on how to control a product during storage and preservation. For this purpose, the optimum water activity $a_{owa}$for conservation was determined. It groups together all the experimental points of the sorption isotherm in a third-degree polynomial equation $X_{eq} = f(a_w)$; thus, it is possible to calculate the optimum relative humidity value for conservation which the second derivative of $X_{eq}$ cancelsand changes sign, so we have an inflection point and the curvature of the curve reverses (Hamza et al., 2020a).It was estimated from the tangent to the graph ($X_{eq} = f(a_w)$) at this value of $a_{owa.}$

**Impact of irrigation systems on hygroscopic parameters**

Fresh samples of saffron grown by three irrigation systems (drip, porous and gravity) were collected and transported to the laboratory where they underwent a controlled adsorption and desorption process at three temperatures (30, 40 and 50 °C) in order to determine their equilibrium moisture content and water activity.

**Analysis of secondary metabolites of saffron by ISO 3632-2 method**

0.05 g of dried stigmas were ground with a porcelain mortar, sieved to 0.5 mm diameter; then, placed in a 100 mL volumetric flask with 90 mL of purified water. The resulting solution was magnetically stirred in the dark for 1 hour and then supplemented with 10 mL of purified water and homogenized. The prepared extracts were filtered through a syringe filter (PTFE, 0.45µm). Then, the absorbance was measured by spectrophotometer (Agilent Cary 60 UV-vis) at the desired wavelength 330 nm for safranal, 440 nm for crocin and 257 nm for picrocrocin. Their contents were expressed by the equation (4).

## Results And Discussions:-

**Sorption isotherms and hysteresis effect**

The experimental data of desorption and adsorption isotherms of *Crocus sativus L.* obtained for the three temperatures of 30, 40 and 50 °C are represented respectively in table 5 and 6. Indeed, at constant water activity, the equilibrium moisture content decreases with increasing temperature. Moreover, the equilibrium water content ($X_{eq}$) increases with increasing of water activity ($a_w$) at a constant temperature.

The phenomenon of hysteresis is illustrated in the Figure 3, it is observed a non-coincidence of the desorption and adsorption curves of *Crocus sativus L.* for the used temperatures where the desorption curve is above that of adsorption.

The isotherms have a sigmoid shape (type II), which is common to many hygroscopic products (Arslan-Tontul, 2021; Jamali et al., 2006). This result can be explained by the fact that the excitation of water molecules increases with increasing temperature; thus reducing the forces of attraction between them, which decreases the equilibrium moisture content (Lahnine et al., 2016b). This result is in line with the hygroscopic behavior of many food products (Arslan-Tontul, 2021; Ouaabou et al., 2021; Tagnamas et al., 2021). The hysteresis effect found in the sorption curves of *Crocus sativus L.* can be explained by the theory that the product's hysteresis is caused by the fact that the deformations that occur during the material's adsorption and desorption don't unfold elastically. In addition, the liquid-solid contact angle is greater when a liquid wets a dry surface (adsorption) than when it withdraws from a wet surface (desorption) (Al-Muhtaseb et al., 2002). As a result, there is an irreversibility in the sorption process. Several researchers in the field of sorption have validated this finding (Machhour et al., 2012; Mohamed et al., 2005).

**Fitting analysis**

Table 7 shows the statistical parameters of the five mathematical models at three temperatures 30, 40 and 50 °C for the sorption isotherms of *Crocus sativus L.* The best model is the one with the highest correlation coefficient $R^2$ and the lowest standard error SE. For our study, the GAB model was chosen as the most appropriate to describe the sorption curves of *Crocus sativus L.* based on SE and $R^2$.

The GAB (Guggenheim - Anderson - De Boer) model has been widely used to describe the hygroscopic behavior of foods (Iglesias et al., 2022). It is applicable to products with an optimum water activity between 0.05 and 0.95 (Al-Khalili et al., 2021). The coefficients of the GAB model have clear thermodynamic explanations that can be useful to better understand the sorption process (Nabily et al., 2020).

**Determination of equilibrium moisture content and optimal water activity**

Figure4 represents the evolution of the equilibrium moisture content of *Crocus sativus L.* as a function of the water activity under the experimental conditions for the saffron sample of the altitude 1700 m. A third-degree polynomial equation of equilibrium moisture content based on water activity is expressed also in Figure 4.

This equation characterizes the stigmas of saffron, it describes the equilibrium water content according to the water activityunder the experimental conditions. It allows a given equilibrium content to estimate the water activity of *Crocus sativus L.* during the drying process until obtaining the optimal water activity for conservation of the product.

The optimum relative humidity value for storage of *Crocus sativus L.* of the sample 1700 m for examplewas calculated using the second derivative of the equation cited in the figure 4 called the "inflection point". The value of the optimum water activity ($a_{owa}$) for conservation of the *Crocus sativus L.* was 0.32 and its corresponding equilibrium water content was 0.64 (% d.b). Equilibrium moisture content is the amount of moisture of a hygroscopic material at its equilibrium. By knowing its values, we can understand how a product loses and gains moisture during storage. Its determination for a product is essential before the drying process. It is also used in the simulation of logarithmic models used in the design and dimensioning of dryers.

Table 8 shows the values of water activity, initial and equilibrium moisture content and humidity obtained for different samples of Moroccan saffron. It was noted that the average value of initial moisture content of Moroccan saffron was 13 % db. While, the average value of its equilibrium moisture content was 0.64 % with 11.72 % as an average value of humidity.

The determination of the water activity for the different samples of Moroccan saffron was carried out in the same way as the sample of altitude 1700 m based on the Figure 4. It is observed from this figure and the table 8 that the water activity of Moroccan safran range between 0.32 and 0.34. These values reflect the availability of water, i.e., the optimal amount of free water which must be contained in the stigmas of saffron in order to limit its deterioration. In effect, microorganisms require "free" water to proliferate and perform biochemical activities, therefore the higher the water activity, the more free water is required and the better they develop.These values is in agreement with the range of optimum water activities found in the literature (0.2-0.4) (Bahammou et al., 2020).This field denotes the area of food product stability where no reaction occurred, such as oxidation, hydrolysis, enzymes, non-enzymatic browning, or microorganism proliferation (Figure 5) (Rockland and Stewart 1981). As a result, saffron stigmas must have a water activity of 32 to 34 percent of relative humidity in order to be preserved and stored for a long time.

Many studies have documented the effect of water activity on changes in food product quality (Gonzalles et al., 2021). In the case of saffron, it has been found that the water activity and saffron's quality are significantly correlated (Hosseini et al., 2018). Crocin, picrocrocin and safranal, which are respectively responsible for saffron'scolor, bitterness, and aroma, are the three principal secondary metabolites that determine the quality of this plant (Lage and Cantrell, 2009). The shelf life of saffron appears to be influenced by the fate of its pigments, the existence of crocin, crocetin (a crocin aglycone generated in biological systems by hydrolysis of crocin as a bioactive metabolite), and other carotenoids (Raina et al., 1996). According to Pfander and Schurtenberger(Pfander and Schurtenberger, 1982), when $a_w$ increased, bitter index values decreased and aroma index values rose concurrently. Tsimidou and Biliadris(Tsimidou and Biliaderis, 1997) have evaluated the kinetics of carotenoid loss during storage of saffron powder at various water activities and temperatures. They discovered that the rate of pigment degradation was accelerated by increasing temperature and relative humidity and was similar to first-order type reactions. This behavior could be explained by the fact that saffron carotenoids are more water soluble than those from other sources, favoring a greater accessibility of dissolved oxygen to the pigments. In fact, nonenzymatic browning, the deterioration of water-soluble pigments and vitamins, and reactions governed by the mobility of reactants in food are all examples of processes whose rates of oxidation of saffron pigments are dependent on moisture content. They further expressed that, at room temperature, medium water activities (0.43-0.53) might more effectively block carotenoid degradation events and boost the development of fragrance components. Based on previous research, the kinetic data for the oxidation of saffron carotenoids reveal an increase in rate with increasing $a_w$ between 0.11 and 0.65. They concluded that the storage conditions used affect the stability of the carotenoids found in saffron. Selim et al. and CP del Campo et al. (del Campo et al., 2010; Selim et al., 2000) discovered the same result, namely that the crocetin esters present in saffron deteriorates as water activity increases. AyhanTopuz has found the same remark for paprika (Topuz, 2008). Topuz has observed that the rate of color deterioration in paprika increased with increasing $a_w$, a finding that was in agreement with what the human eye saw. This color degradation can also be brought on by non-enzymatic browning reactions which are closely correlated to the water activity of paprika or by hydrolysis of the fatty acid ester.Paprika's color was discovered to be more stable with low water activity. The impact of water activity on the stability of carotenoids in dried carrots has also been investigated by Lavelli et al. (Lavelli et al., 2007). They have found that carotenoids in dried carrots were shown to be more stable at $a_w$ of 0.32-0.57.

Hosseini et al. (Hosseini et al., 2018) have also declared that the preservation of crocin and picrocrocin in saffron requires low temperatures and low relative humidity. They added that the decomposition of saffron carotenoid (mostly crocin) is reliant on water activity, temperature, light, pH and oxygen. As a result, oxidative degradation of the saffron pigments increased as water activity increased. The best storage conditions for saffron should include a dark, inert environment, a lower temperature (< 25 °C) and low water activity level (< 0.43). The shelf life will be longer than 3 years if the saffron stigma is stored at $a_w$ = 0.32 and in the dark.

According to these studies, it can be concluded that the saffron used in our study maintains its carotenoids (particularly crocin and crocetin) and retains its organoleptic qualities (color, bitterness, and aroma) in its optimal water activity of storage and conservation found between 0.32 and 0.34, which corresponds to its zone of physico-chemical stability, and its drying must adhere to the conditions mentioned in this study (30 °C <T< 50 °C).

**Impact of irrigation system on equilibrium moisture content and water activity**

As demonstrated in Table 9, the moisture content on a dry basis was varied as function of temperature, while the water activity was depended on the irrigation systems for a fresh sample of saffron. It is noticeable that the samples irrigated by the gravity system are very wet (86% water), followed by those irrigated by the porous system (77%) and the drip system (75%). It is also noticed that the equilibrium moisture content depends on the sorption temperature. Indeed, it decreases progressively with the increase of the temperature. Thus, its highest values are observed in the gravity system. We also added that the water activity depends on irrigation system as illustrated in Table 9. the irrigation with a gravity system gives a saffron with a water activity of 0.34, while the saffron irrigated with drip system has a water activity of 0.32.

If irrigation is regular and done outside of the peak evapotranspiration hours, the soil's water content always exceeds the plant's need because the gravity system only distributes water superficially and submerges the plant at the root portion. The saffron bulbs that are watered by this technique are located in moist soils. Since saffron grows effectively in silty, sandy, well-draining soils, it is clear that the hydrous retention of the vegetable matter is also crucial.

Additionally, the porous subsoil system places the bulbs in moist soils and provides them with shelter. The saffron produced by this system is very humid but ranks better than that of the gravity system.

On the contrary, the drip system is a superficial system that is qualified by the optimization and localization of irrigation. This peculiarity acquires a rational quantity of water for the plant and its benefit in its almost totality and without the risk of being damaged. In this case, the stigmas of the saffron retain less water than plant matter compared to the other two systems.

For all three temperatures, the temperature of 40°C is more moderate so that the plant cell will exude its water for a shorter time compared to the 30°C and 50°C temperatures. And it is congruent on the saffron produced by the three irrigation systems and which presents an increasing hydric potential respectively of the drip, porous and gravitational.

**Effect of altitudes on the content of the main compounds of Moroccan saffron**

Saffron samples were analyzed according to ISO 3632-2. This method allows the determination of the main characteristics of saffron related to picrocrocin, safranal and crocin. Figures 6, 7 and 8 present the calibration curves used for determination of the concentrations of these molecules with a regression coefficient ($R^2$) greater than 0.99.

Table 10 represents the experimental results obtained. It is noticed that the parameter of the altitude didn’t have anexpressif influence on the content of safranal, crocin and picrocrocin. The highest values of safranal were obtained for the samples of 1000 m (43.92 $\mathbf{E}_{330}^{1\%}$) and 2100 m (41.47 $\mathbf{E}_{330}^{1\%}$) and the highest values of crocin were obtained for the samples of 1700 m (291.68 $\mathbf{E}_{440}^{1\%}$) and 1600 m (258.03 $\mathbf{E}_{440}^{1\%}$),while the minimum content of crocin and safranal was observed for the 1500 m sample’s. For the picrocrocin, its highest value was obtained for the sample of 1700 m (120.11 $\mathbf{E}_{257}^{1\%}$),and its minimum content was found for the 1000 m sample’s.

According to ISO 3632-1, all of the altitudes utilized in this study were categorized as category 1 and 2 (Table 10). The samples from altitude 1700 m recorded high concentrations of crocin (291.68$\mathbf{E}_{440}^{1\%}$ ) and picrocrocin (120.11$\mathbf{E}_{257}^{1\%}$ ).

This difference of quality between the samples of saffron can be explained by the particularity that characterizes each site (each altitude) namely: the geographical origin, the environmental and climatic conditions, the method and the climate of the harvest as well as the technique of drying used (Lage and Cantrell, 2009).

**Tables**

**Table 1:-** Climatic and geographical conditions of the sampling areas.

| N° | Communes | Coordinates Lumbar | Altitudes | Temperature | Time | Humidity | Wind air velocity (Km/h) |
|---|---|---|---|---|---|---|---|
| 1 | Assaiss-Tizgui | N30.6259,08° W7.576153° | 2100 | 7 °C | 07 : 00 | 45 % | 11.8 |
| 2 | Assaiss-AitIicht | N30.563208 W7.622843° | 1700 | 8 °C | 07 :10 | 43 % | 11.8 |
| 3 | Assaiss-Dogadir | N30.474062° W7.740737° | 1600 | 8 °C | 07 :15 | 40 % | 11.7 |
| 4 | Tassoussfi-Awrest | N30.254960° W7.535762° | 1500 | 10 °C | 06 :45 | 35 % | 11.9 |
| 5 | Tassoussfi-Souktana | N30.524302° W7.889155° | 1200 | 11 °C | 06 :30 | 32 % | 11.9 |
| 6 | Taliouine-Taghzout | N30.530138° W7.910317° | 1000 | 12 °C | 06 :30 | 29 % | 12.0 |

**Table 2:-** Saturated salt solutions and their relative humidity at three temperatures.

| | KOH | $MgCl_2, 6H_2O$ | $K_2CO_3$ | $NaNO_3$ | KCl | $BaCl_2, 2H_2O$ |
|---|---|---|---|---|---|---|
| 30 ° C | 7.38 | 32.38 | 43.17 | 72.75 | 83.62 | 89.80 |
| 40 ° C | 6.26 | 31.59 | 42.30 | 71.00 | 82.32 | 89.10 |
| 50 ° C | 5.72 | 30.54 | 40.91 | 69.04 | 81.20 | 88.23 |

**Table 3:-** Summary of equations used for data treatment.

| **Equations** | **Legend** | **References** | **N°** |
|---|---|---|---|
| $EMC = X_{eq} = \frac{M_w - M_d}{M_d}$ | $X_{eq}$ – equilibrium moisture content<br>$M_w$ - equilibrium mass of the sample (kg)<br>$M_d$ - dry mass of the sample (kg). | (Bahammou et al., 2020) | **(1)** |
| $R^2 = \sqrt{\frac{\sum_{i=1}^{N}(X^*_{eq_{i,pred}} - \overline{X}^*_{eq_{i,\exp}})^2}{\sum_{i=1}^{N}(X^*_{eq_{i,\exp}} - \overline{X}^*_{eq_{i,\exp}})^2}}$ | $X^*_{eq_{i,pred}}$ - $i^{ème}$ moisture ratio predicted by model.<br>$X^*_{eq_{i,\exp}}$ - $i^{ème}$ experimental moisture ratio. | (Oubella et al., 2022) | **(2)** |
| $SE = \sqrt{\sum_{i=1}^{N}\frac{\left(X^*_{eq_{i,\exp}} - X^*_{eq_{i,pred}}\right)^2}{f}}$ | N - number of experimental data.<br>f- degree of freedom of the regression model. | (Oubella et al., 2022) | **(3)** |
| $E^{1\%}_{1cm}\ \lambda_{\max} = \frac{A \times 10000}{m \times (100 - H)}$ | $E^{\%}$-the molecular extinction coefficient of a 1% (w/v) solution in a path length of 1 cm.<br>A- the absorbance values<br>m - the weight of the sample (g)<br>H - the moisture and volatile content estimated at 103 ± 2 °C for 16h. The amount of H in this study was 7.25%. | (Lage and Cantrell, 2009) | **(4)** |

**Table 4:-** Mathematical models applied to sorption isotherms of *Crocus sativus L.*

| **Model name** | **Equation** | **N°** |
|---|---|---|
| **GAB** | $X_{eq} = \frac{ABCa_w}{[1-Ba_w][1-Ba_w+BCa_w]}$ | **(5)** |

| | | |
|---|---|---|
| **Modified Oswin** | $X_{eq} = (A+B\theta)(a_w)^C / (1-a_w)^C$ | **(6)** |
| **Smith** | $X_{eq} = A + B\log(1-a_w)$ | **(7)** |
| **Modified Henderson** | $X_{eq} = \left(-\ln(1-a_w)/A(\theta+B)\right)^{1/C}$ | **(8)** |
| **Peleg** | $X_{eq} = A(a_w)^C + B(a_w)^D$ | **(9)** |

A, B, C, and Dare the model coefficients and θ (°C) is the sorption temperature.

**Table 5:-** Desorption isotherms data of *Crocus sativus L.* under experimental conditions.

| **30 °C** | | **40 °C** | | **50 °C** | |
|---|---|---|---|---|---|
| **Water activity $a_w$ (-)** | **Equilibriummoisture content $X_{eq}$ (% d.b)** | **Water activity $a_w$ (-)** | **Equilibriummoisture content $X_{eq}$ (% d.b)** | **Water activity $a_w$ (-)** | **Equilibriummoisture content $X_{eq}$ (% d.b)** |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0738 | 0.66722 | 0.0626 | 0.42808 | 0.0572 | 0.3876 |
| 0.3238 | 0.70423 | 0.3159 | 0.50314 | 0.3054 | 0.43384 |
| 0.4317 | 0.89943 | 0.423 | 0.70312 | 0.4091 | 0.65488 |
| 0.7275 | 1.71875 | 0.71 | 1.67683 | 0.6904 | 0.93516 |
| 0.8362 | 2.78481 | 0.8232 | 2.5 | 0.812 | 2.32095 |
| 0.898 | 3.97351 | 0.891 | 2.96717 | 0.8823 | 2.48756 |

**Table 6:-** Adsorption isotherms data of *Crocus sativus L.* under experimental conditions.

| **30 °C** | | **40 °C** | | **50 °C** | |
|---|---|---|---|---|---|
| **Water activity $a_w$ (-)** | **Equilibriummoisture content $X_{eq}$ (% d.b)** | **Water activity $a_w$ (-)** | **Equilibriummoisture content $X_{eq}$ (% d.b)** | **Water activity $a_w$ (-)** | **Equilibriummoisture content $X_{eq}$ (% d.b)** |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0738 | 0.57859 | 0.0626 | 0.33445 | 0.0572 | 0.27285 |
| 0.3238 | 0.67237 | 0.3159 | 0.42513 | 0.3054 | 0.38204 |
| 0.4317 | 0.78563 | 0.423 | 0.70312 | 0.4091 | 0.48426 |
| 0.7275 | 1.52068 | 0.71 | 1.44262 | 0.6904 | 0.92421 |
| 0.8362 | 2.67966 | 0.8232 | 2.51799 | 0.812 | 2.2118 |
| 0.898 | 3.37079 | 0.891 | 2.93413 | 0.8823 | 2.4 |

**Table 7:-** Statistical parameters for the 5 mathematical models describing the sorption isotherms of *Crocus sativus L.* at three temperatures.

| **Model** | **Parameters** | **Desorption** | | | **Adsorption** | | |
|---|---|---|---|---|---|---|---|
| | T (°C) | **30** | **40** | **50** | **30** | **40** | **50** |
| **GAB** | A | 0.6244 | 0.9391 | 0.4570 | 0.5032 | 0.8571 | 0.9503 |
| | B | 0.6969 | 0.8495 | 0.9514 | 0.9512 | 0.8495 | 0.9760 |
| | C | $3.00\ 10^2$ | 2.1935 | $1.45\ 10^2$ | $1.81\ 10^7$ | 1.8708 | $1.12\ 10^2$ |
| | **$R^2$** | **0.9992** | **0.9918** | **0.9698** | **0.9958** | **0.9914** | **0.9763** |
| | **SE** | **0.0700** | **0.1016** | **0.2932** | **0.1385** | **0.1834** | **0.2645** |
| **Modified Oswin** | A | $8.51\ 10^4$ | $-5.53\ 10^4$ | $7.69\ 10^4$ | $3.22\ 10^4$ | $1.46\ 10^5$ | $7.12\ 10^5$ |
| | B | $2.84\ 10^3$ | $1.38\ 10^3$ | $1.53\ 10^3$ | $-0.71\ 10^2$ | $-3.66\ 10^3$ | $-1.42\ 10^3$ |
| | C | 0.5930 | 0.5364 | 0.5892 | 0.5575 | 0.5814 | 0.6405 |
| | $R^2$ | 0.9901 | 0.9904 | 0.9666 | 0.9890 | 0.9894 | 0.9736 |
| | SE | 0.2431 | 0.1920 | 0.3123 | 0.2227 | 0.2033 | 0.2729 |
| **Smith** | A | 0.0121 | 0.0929 | 0.0765 | 0.1271 | 0.0032 | 0.1324 |
| | B | -0.1540 | -0.3149 | -0.3246 | -1.3569 | -1.3158 | -1.1300 |
| | $R^2$ | 0.9785 | 0.9928 | 0.9636 | 0.9824 | 0.9902 | 0.9709 |
| | SE | 0.3245 | 0.1506 | 0.2900 | 0.2518 | 0.1747 | 0.2534 |
| **Modified** | A | $1.52\ 10^1$ | $5.92\ 10^1$ | $1.43\ 10^2$ | $3.54\ 10^1$ | $7.07\ 10^1$ | $7.89\ 10^1$ |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Henderson** | B | $2.18\ 10^3$ | $4.82\ 10^3$ | $5.22\ 10^3$ | $2.34\ 10^3$ | $5.58\ 10^3$ | $5.69\ 10^3$ |
| | C | $3.90\ 10^1$ | $5.98\ 10^1$ | $3.19\ 10^2$ | $4.68\ 10^1$ | $5.95\ 10^1$ | $6.87\ 10^1$ |
| | $R^2$ | 0.9764 | 0.9910 | 0.9623 | 0.9795 | 0.9900 | 0.9709 |
| | SE | 0.3736 | 0.1907 | 0.3309 | 0.3031 | 0.2003 | 0.2850 |
| **Peleg** | A | -0.3175 | -0.8521 | $-1.21\ 10^2$ | -0.3081 | -0.0945 | $-1.09\ 10^2$ |
| | B | 0.3635 | 0.8882 | $1.21\ 10^2$ | 0.3475 | 0.1314 | $1.09\ 10^2$ |
| | C | 2.3432 | 1.9330 | 2.2483 | 2.1428 | 2.1756 | 2.5793 |
| | D | 2.3432 | 1.9330 | 2.2483 | 2.1428 | 2.1756 | 2.5793 |
| | $R^2$ | 0.9533 | 0.9856 | 0.9514 | 0.9595 | 0.9839 | 0.9688 |
| | SE | 0.6087 | 0.2734 | 0.4312 | 0.4901 | 0.2913 | 0.3402 |

**Table 8:-** Water activities and equilibrium moisture contents of different samples of Moroccan saffron during sorption isotherms.

| N° | Water activity | Initial moisture content (% db) | Equilibriummoisture content (% db) | Humidity (%) |
|---|---|---|---|---|
| 1 | 0.34 | 15 | 0.63 | 12.86 |
| 2 | 0.32 | 13 | 0.64 | 11.81 |
| 3 | 0.32 | 14 | 0.64 | 12.50 |
| 4 | 0.33 | 12 | 0.63 | 10.47 |
| 5 | 0.32 | 11 | 0.64 | 9.92 |
| 6 | 0.33 | 15 | 0.63 | 12.78 |

**Table 9:-** Dry basis equilibrium moisture content at different temperatures for the three irrigation systems with water activities values.

| | | **Water activity** | **Initial moisture content (% db)** | **Equilibrium moisture content (% db)** | **Humidity (%)** |
|---|---|---|---|---|---|
| **Drip system** | 30 °C | 0.32 | 2.95 | 0.39 | 75 |
| | 40 °C | | 2.95 | 0.09 | 75 |
| | 50 °C | | 2.94 | 0.01 | 75 |
| **Gravity system** | 30 °C | 0.34 | 5.95 | 1.64 | 86 |
| | 40 °C | | 6.16 | 0.29 | 86 |
| | 50 °C | | 6.10 | 0.24 | 86 |
| **Porous system** | 30 °C | 0.33 | 3.28 | 0.44 | 77 |
| | 40 °C | | 3.27 | 0.19 | 77 |
| | 50 °C | | 3.27 | 0.16 | 77 |

**Table 10:-** Comparison of the values of E1% of safranal, crocin and picrocrocin obtained by the procedure ISO 3632-2 for different aeras of Moroccan saffron and their classification.

| **Altitudes** | **ISO 3632-2** | | | **Classification of ISO 3632-1** |
|---|---|---|---|---|
| | **UV-vis** | | | |
| | $\mathbf{E_{330}^{1\%}}$ | $\mathbf{E_{440}^{1\%}}$ | $\mathbf{E_{257}^{1\%}}$ | |
| 1000 m | 43.92 | 164.55 | 82.04 | Category II |
| 1200 m | 36.75 | 202.38 | 90.00 | Category I |
| 1500 m | 30.97 | 158.71 | 77.25 | Category II |
| 1600 m | 35.45 | 258.03 | 110.32 | Category I |
| 1700 m | 38.60 | 291.68 | 120.11 | Category I |
| 2100 m | 41.47 | 181.49 | 83.45 | Category II |

**Figures**

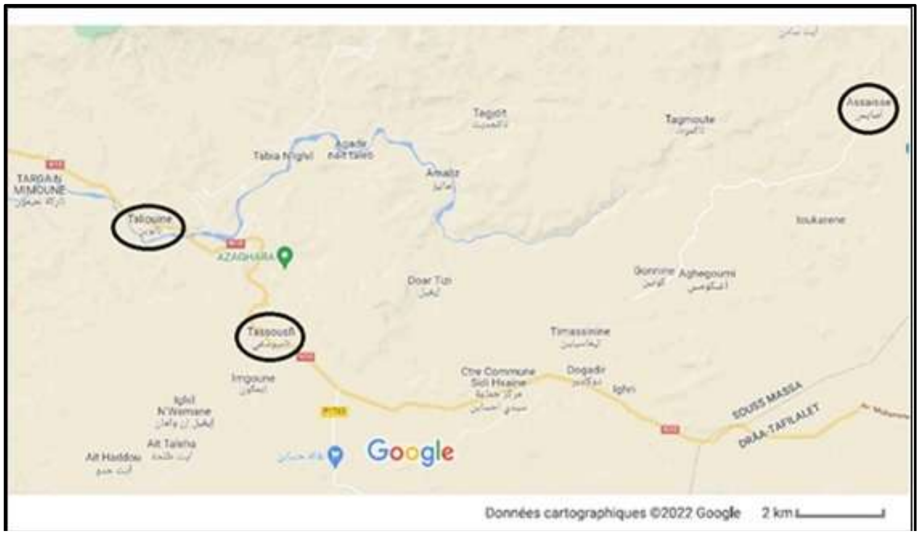


**Figure 1:-** Geographic map of sampling areas.

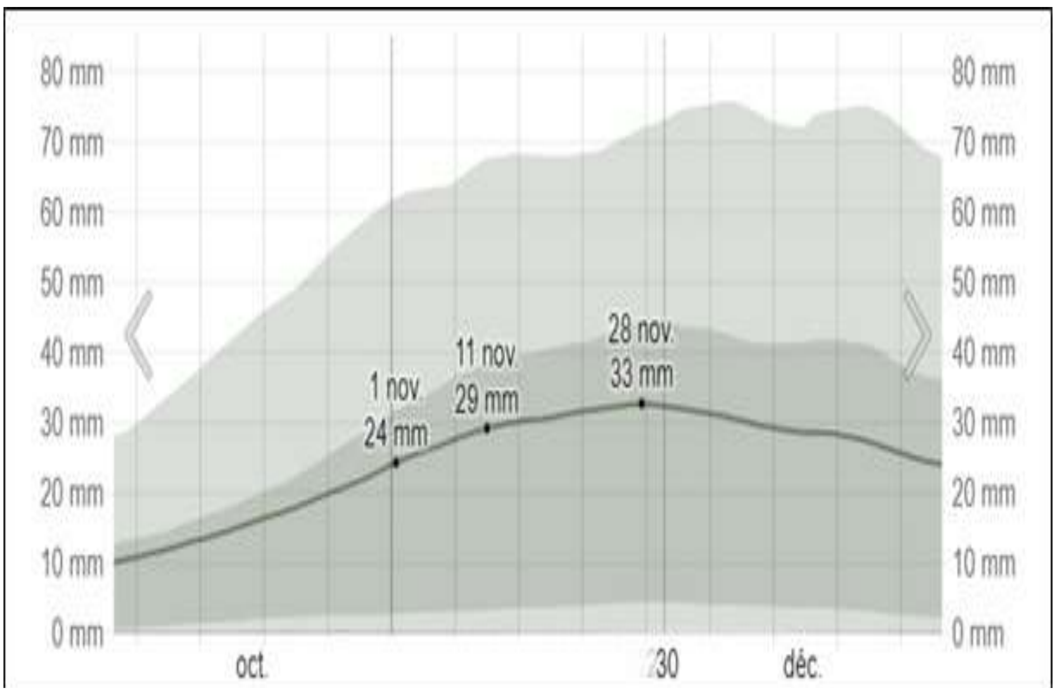


**Figure 2:-** Average rainfall in November for the Taliouine region.

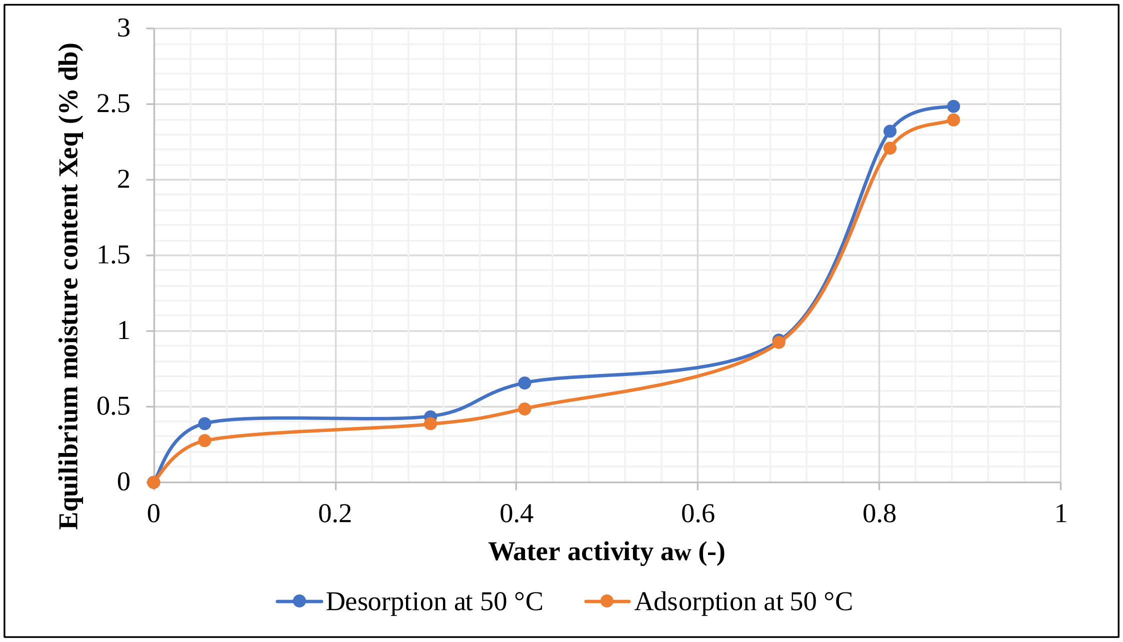


**Figure 3:-** Hysteresis phenomenon of *Crocus sativus L.* at 50 °C.

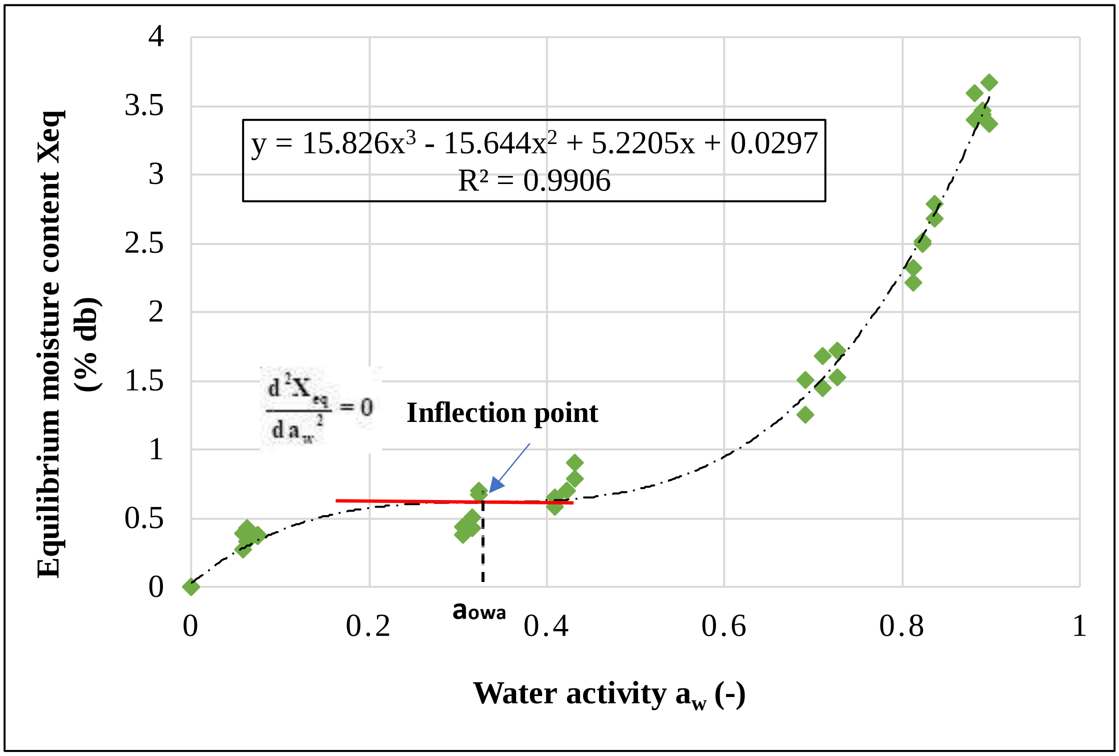


**Figure 4:-** Example of determination of optimal water activity for *Crocus sativus L.* of 1700 m.

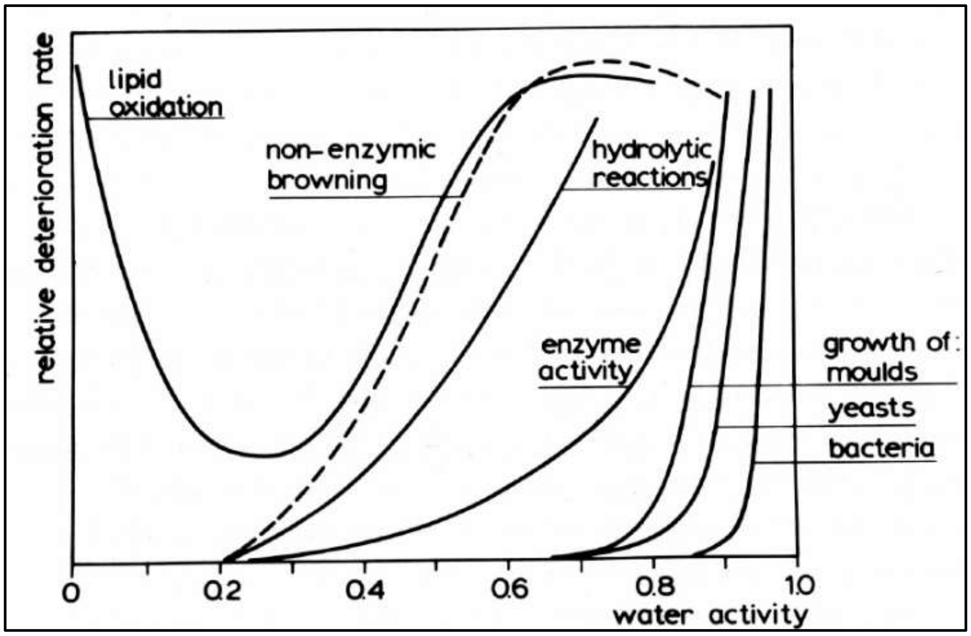


**Figure 5:-** Reaction rates in food as function of water activity.

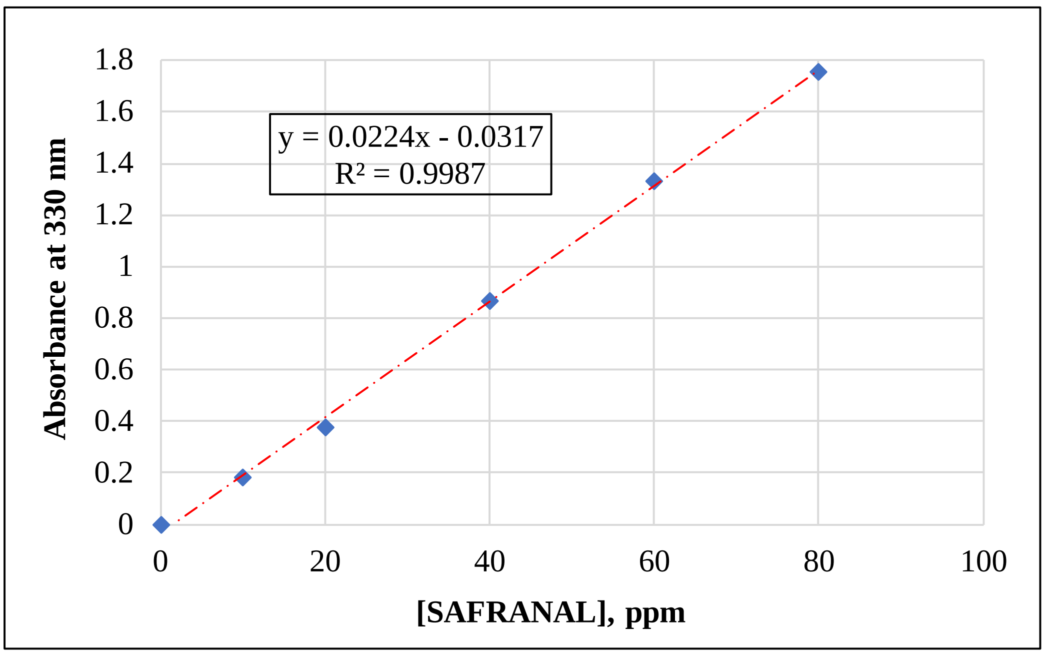


**Figure 6:-** Calibration curve-Safranal-Extraction with pure water - UV-vis.

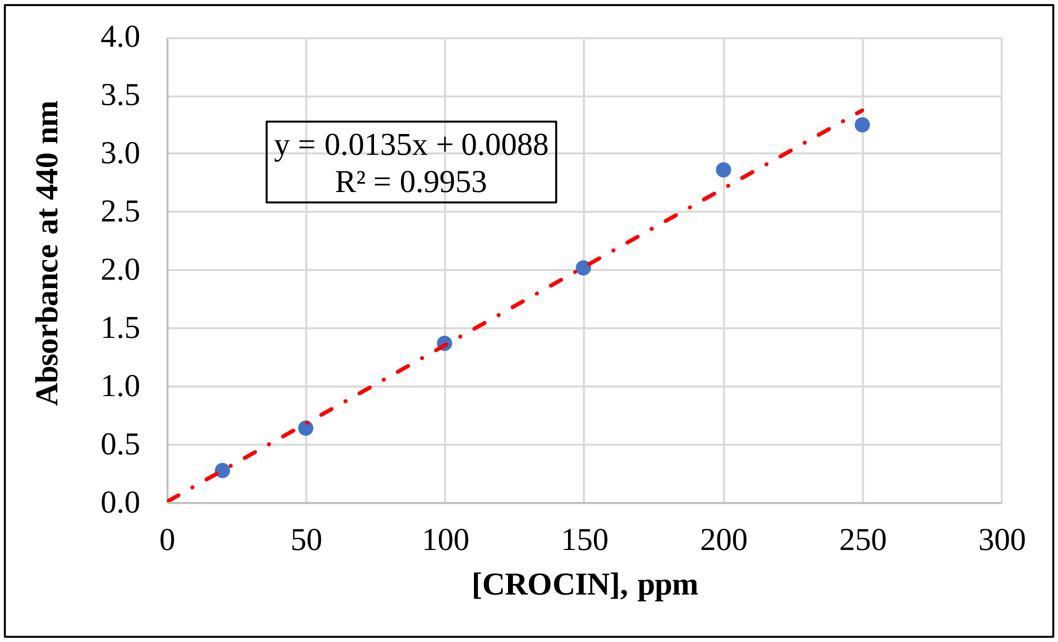


**Figure 7:-** Calibration curve-Crocin-Extraction with pure water - UV-vis.

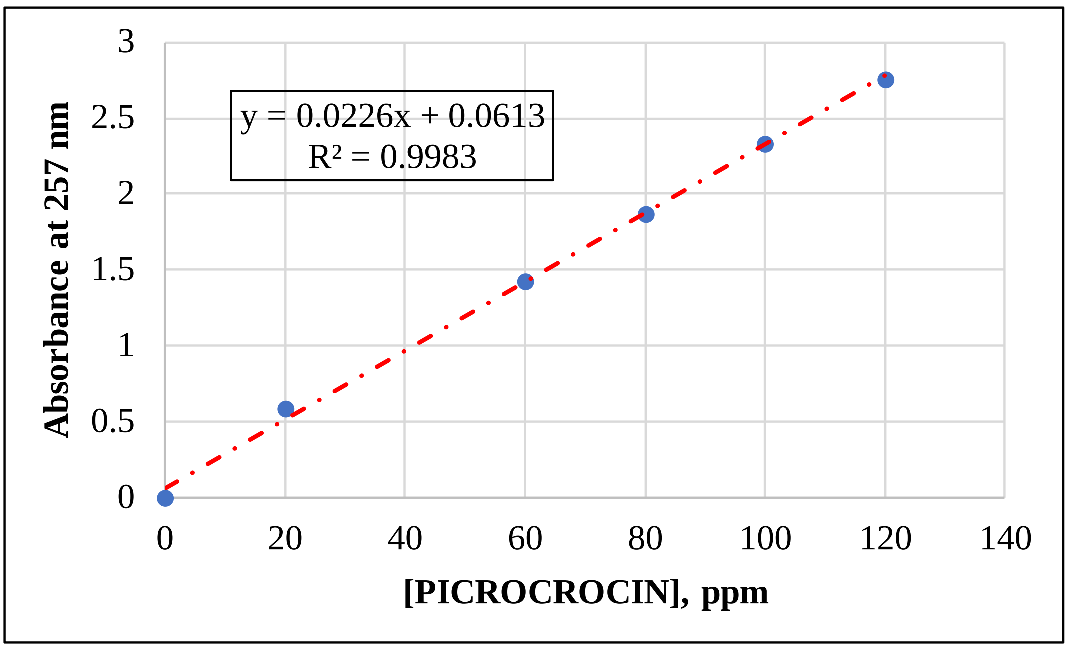


**Figure 8:-** Calibration curve-Picrocrocin-Extraction with pure water - UV-vis.

## Conclusion:-

The effect of the altitude on the water activity and the composition of bioactives molecules of the Moroccan saffron was well noticed in this study. The results of the hygroscopic analysis carried out for the six samples of saffron resulting from various zones of Taliouine region showed that the optimal water activity for conservation of this spice was ranged between 0.32 and 0.34. This field indicates the region of food product stability where no reaction had place, such as oxidation, hydrolysis, enzymes, non-enzymatic browning, or microbial growth. The measurement of water activity thus guarantees a consistently high product quality. The impact of irrigation systems on the water activity showed that drip system offers a saffron with a minimum value of water activity up to 0.32. The quality analysis revealed that the Moroccan saffron had a good quality since it is situated between the category I and II of the ISO classification.

It is now recognized that sorption data provide more complete information than a simple determination of water activity. The hygroscopic study of a product is an essential and indispensable step in the pharmaceutical and food industry, since it gives us information on the optimal preservation conditions of the product.

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## References:-

1. Al-Khalili M., Al-Habsi N., Rahman M.S. (2021): Application of Dynamic Temperature-Humidity Chamber for Measuring Moisture Sorption Isotherms of Biomaterials as Compared to the Conventional Isopiestic Method. Adsorption Science & Technology, 2021: 1–13. https://doi.org/10.1155/2021/1236427
2. Al-Muhtaseb A.H., McMinn W.A.M., Magee T.R.A. (2002): Moisture Sorption Isotherm Characteristics of Food Products: A Review. Food and Bioproducts Processing, 80:118–128. https://doi.org/10.1205/09603080252938753
3. Arslan-Tontul S. (2021): Moisture sorption isotherm and thermodynamic analysis of quinoa grains. Heat Mass Transfer, 57: 543–550. https://doi.org/10.1007/s00231-020-02978-8
4. Azhar M.D., Abd Hashib S., Ibrahim U.K., Md Zaki N.A., Ahmad ZamanhuriN., Abd Rahman N. (2021): Moisture sorption isotherm and thermodynamic properties of Centella asiatica L. (CAL) powder. Chemical Engineering Communications, 208: 573–582. https://doi.org/10.1080/00986445.2020.1780213
5. Bahammou Y., Moussaoui H., Lamsayeh H., Tagnamas Z., Kouhila M., Ouaabou R., Lamharrar A., Idlimam A.(2020): Water sorption isotherms and drying characteristics of rupturewort (Herniariahirsuta) during a convective solar drying for a better conservation. Solar Energy, 201: 916–926.https://doi.org/10.1016/j.solener.2020.03.071
6. CardoneL., Castronuovo D., Perniola M., Cicco N., Candido V. (2020): Saffron (Crocus sativus L.), the king of spices: An overview. Scientia Horticulturae, 272:109560. https://doi.org/10.1016/j.scienta.2020.109560
7. del Campo C.P., Carmona M., Maggi L., Kanakis C.D., Anastasaki E.G., Tarantilis P.A., Polissiou M.G., Alonso G.L. (2010): Effects of mild temperature conditions during dehydration procedures on saffron quality parameters: Effects of mild temperature conditions on saffron quality parameters. J. Sci. Food Agric. 90: 719–725. https://doi.org/10.1002/jsfa.3877
8. Gonzalles G., Geng N., Luo S., Zhang C., Wu C., Li D., Li Y., Song J. (2021): Effects of different water activities on the stability of carotenoids in puff-dried yellow peach powder during storage. qas 13: 1–8. https://doi.org/10.15586/qas.v13SP2.944
9. HamzaL., Mounir K., Younes B., Zakaria T., Haytem M., Hind M., Abdelkader L., Ali I.(2020): Physicochemical study of the conservation of Moroccan anchovies by convective solar drying. Renewable Energy 152: 44–54. https://doi.org/10.1016/j.renene.2020.01.039
10. HosseiniA., Razavi B.M., Hosseinzadeh H. (2018): Pharmacokinetic Properties of Saffron and its Active Components. Eur J Drug MetabPharmacokinet 43: 383–390. https://doi.org/10.1007/s13318-017-0449-3
11. Iglesias H.A., Baeza R., Chirife J. (2022): A Survey of Temperature Effects on GAB Monolayer in Foods and Minimum Integral Entropies of Sorption: a Review. Food Bioprocess Technol. https://doi.org/10.1007/s11947-021-02740-w
12. JamaliA., Kouhila M., MohamedL.A., Idlimam A., Lamharrar A. (2006): Moisture adsorption–desorption isotherms of Citrus reticulata leaves at three temperatures. Journal of Food Engineering 77: 71–78. https://doi.org/10.1016/j.jfoodeng.2005.06.045
13. Jeantet R., Croguennec T., Schuck P., Brulé G. (2006): Science des aliments: biochimie, microbiologie, procédés, produits. Londres Éd. Tec & doc, Paris New York.
14. Kane C.S.E., Kouhila M., Lamharrar A., Idlimam A., Mimet A. (2008): Moisture sorption isotherms and thermodynamic properties of tow mints: Mentha pulegium and Mentha rotundifolia 15.
15. LageM., Cantrell C.L. (2009): Quantification of saffron (Crocus sativus L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. Scientia Horticulturae 121: 366–373. https://doi.org/10.1016/j.scienta.2009.02.017
16. Lahnine L., Idlimam A., Mahrouz M., Jada A., Hanine H., Mouhib M., Zantar S., Kouhila M. (2016a): Adsorption measurements and modeling of thyme treated with gamma irradiation and thermal–biochemical treatment. Industrial Crops and Products 88: 36–43. https://doi.org/10.1016/j.indcrop.2016.02.049

17. Lahnine L., Idlimam A., Mahrouz M., MghazliS., HidarN., Hanine H., Koutit A. (2016b): Thermophysical characterization by solar convective drying of thyme conserved by an innovative thermal-biochemical process. Renewable Energy 94: 72–80. https://doi.org/10.1016/j.renene.2016.03.014
18. Lavelli V., Zanoni B., Zaniboni A. (2007): Effect of water activity on carotenoid degradation in dehydrated carrots. Food Chemistry 104:1705–1711. https://doi.org/10.1016/j.foodchem.2007.03.033
19. Machhour H., Idlimam A., Mahrouz M., Hadrami I.E., Kouhila M. (2012): Sorption isotherms and thermodynamic properties of peppermint tea (Mentha piperita) after thermal and biochemical treatment 16.
20. Mallek-Ayadi S., Bahloul N., Kechaou N. (2020): Mathematical modelling of water sorption isotherms and thermodynamic properties of Cucumis melo L. seeds. LWT 131: 109727. https://doi.org/10.1016/j.lwt.2020.109727
21. Menghini L., Leporini L., Vecchiotti G., Locatelli M., Carradori S., Ferrante C., Zengin G., Recinella L., ChiavaroliA., Leone S., Brunetti L., Orlando G. (2018): Crocus sativus L. stigmas and byproducts: Qualitative fingerprint, antioxidant potentials and enzyme inhibitory activities. Food Research International 109: 91–98. https://doi.org/10.1016/j.foodres.2018.04.028
22. MohamedL.A., Kouhila M., Lahsasni S., Jamali A., Idlimam A., Rhazi M., Aghfir M. Mahrouz M. (2005): Equilibrium moisture content and heat of sorption of Gelidiumsesquipedale. Journal of Stored Products Research 41: 199–209. https://doi.org/10.1016/j.jspr.2004.03.001
23. Nabily M., Nabili A., Namsi A., Majdoub H., Azzouna A. (2020): Shelf Life Prediction and Storage Stability of Deglet Nour Dates (Phoenix dactylifera L.): Microbiological and Organoleptic Properties. Chemistry Africa 3: 189–197. https://doi.org/10.1007/s42250-019-00108-4
24. Ouaabou R., Ennahli S., Di Lorenzo C., Hanine H., Bajoub A., Lahlali R., Idlimam A., Ait Oubahou A., Mesnaoui M. (2021): Hygroscopic Properties of Sweet Cherry Powder: Thermodynamic Properties and Microstructural Changes. Journal of Food Quality 2021: 1–11. https://doi.org/10.1155/2021/3925572
25. Oubella K., Mouhanni H., Bahammou Y., Idlimam A., Lamharrar A., Bendou A. (2022): Influence of Drying Temperature on the Different Thermodynamic Parameters during the Indirect Convective Solar Drying of Crocus sativus L. Of Morocco Thin-Layer Solar Drying of Moroccan Saffron. The Scientific World Journal 2022: 1656862. https://doi.org/10.1155/2022/1656862
26. Pfander H., Schurtenberger H. (1982) Biosynthesis of C20-carotenoids in Crocus sativus. Phytochemistry 21: 1039–1042. https://doi.org/10.1016/S0031-9422(00)82412-7
27. Raina B.L., Agarwal S.G., Bhatia A.K., Gaur G.S. (1996) Changes in Pigments and Volatiles of Saffron ( Crocus sativusL) During Processing and Storage. J. Sci. Food Agric. 71: 27–32.
28. Rockland L.B. and Stewart G.F. (1981): Water activity: Influences on food quality. In Berg, C. and Bruin, S. eds. Water activity and its estimation in food systems: Theoretical aspects, 1-62. New York: Academic Press.
29. Selim K., Tsimidou M., Biliaderis C.G. (2000): Kinetic studies of degradation of saffron carotenoids encapsulated in amorphous polymer matrices. Food Chemistry 71: 199–206. https://doi.org/10.1016/S0308-8146(00)00156-4
30. Tagnamas Z., Bahammou Y., Moussaoui H., Kouhila M., Lamsyehe H., Idlimam A.,Lamharrar A. (2021): Exploring the sorption and thermodynamic proprieties of white truffle ( Terfeziaboudieri ). J Food Process Eng, 44. https://doi.org/10.1111/jfpe.13888
31. TopuzA. (2008): A novel approach for color degradation kinetics of paprika as a function of water activity. LWT - Food Science and Technology 41: 1672–1677. https://doi.org/10.1016/j.lwt.2007.10.004
32. Tsimidou M., Biliaderis C.G. (1997): Kinetic Studies of Saffron (Crocus sativus L.) Quality Deterioration. J. Agric. Food Chem. 45: 2890–2898.