EFFECT OF THE INCORPORATION SPIRULINA ON THE PHYSICOCHEMICAL PARAMETERS OF A GRAPE JUICE

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Abstract: *Our study focuses on the valuation of Spirulina, which can contain up to 70% of protein, in the manufacture of a dietary product. It aims to elaborate a hyper-protein energy drink of grape juice having a significant nutritional intake .Our study aims to show the effect of the incorporation of spirulina on the physicochemical parameters of a grape juice at different doses (0.1g/l - 0.3g/l - 0.5g/l - 0.7g/l) compared to a control drink and evaluation of its nutritional value. Several analyzes were carried out The Physico-chemical analyzes - acidity, Brix, pH, and density with reference to Algerian standards, as well as sensory analyzes ;stability testing .The statistical analysis (Newman-keuls test) showed that they are not significantly different between all parameters at the probability 0.05 probability level. The physico-chemical analyses obtained from different products are in agreement with Algerian standards.Indeed, the juice has a certain richness in proteins (31%); it is therefore considered a high protein product. A good microbiological quality of the juice was revealed. The F3 formulation (0.5g) is judged to be good, and well appreciated by the tasting panel. Its stability test for 4 weeks at 22°C showed that formulation F3 (0.5g) is a stable juice. There is a significant effect ($p < 100$) of the dose of spirulina incorporated on the parameters studied (Acidity, pH, Density, refractive index). The enrichment of grape juice by spirulina allowed us to obtain a dietetic grape juice, a stable and hyper protein with a protein level of 31%.*

Keywords*: Enrichment, Grape juice, Spirulina, Stability test, Nutritional value.*

1. Introduction

The food industry has shown interest in the application of ultrasound to improve the chemical, physical properties of various foods (Higuera-Barraza et al., 2016). Ultrasound-assisted extraction (UAE) is efficient in the disruption and extraction of algal cells without the addition of beads (for milling) or chemicals, with less energy consumption at relatively lower temperatures leading to less thermal protein denaturation. Also, UAE offers a great reduction in time and solvent consumption (Picó, 2013).

Many authors studied the effect of ultrasound on protein extraction. The use of ultrasound increases the concentration of proteins of Spirulina. Another study shows the positive effect of sonication time and sonication energy on recovery protein yield. The application of Surface Response methodology in the

optimization of analytical procedures is very beneficial due to the generation of a large amount of information from a small number of experiments and the possibility to evaluate the interaction effect between variables on the response (Bezerra et al., 2008).

Spirulina is a great source of natural protein (about 60% digestible proteins). It is low fat, low calorie, cholesterol-free source of protein with all amino acids, phytonutrients, antioxidants, carbohydrates, mucopolysaccharides, itamins, and trace minerals. It is a ubiquitous organism that was used as food in Mexico 400 years ago during the Aztec civilization (Al-Dhabi, 2013).

Spirulina is a freshwater cyanobacterium characterized by a blue-green color due to the presence of a rare protein pigment. It is considered an unconventional food resource that can contain up to 70% of its dry weight, twice as much soy protein, and three times more than beef (Higuera-Barraza et al., 2016).

The spirulina protein supplies the body with almost all essential amino acids, making it an essential food for vegetarians.

Spirulina is used as a dietary supplement, which can be used with all the food and drink we consume. We tried to develop spirulina in the manufacture of a food product namely high protein grape juice.

Fruit juices, derived from a wide range of fruit, including oranges and another citrus, apple, grape, pineapple, and mango, often result from the fruit juice blend of several species. Usually, fruits are processed into concentrates near their harvesting place and then shipped to a conditioner.

The grape juice is an unfermented but fermentable juice, intended for consumption directly and obtained by a mechanical process from sound and ripe grapes exclusively preserved by a physical process.

The juice may have been concentrated and reconstituted with suitable water to preserve the essential compositional factors and the quality of the juice (Picó, 2013).

Our research consists of a comparative study between two grape drinks (one enriched with spirulina and another non-enriched drink) and an evaluation of its nutritional value with enrichment with different doses of spirulina (0.1 g /L - 0.3 g /L -0.5 g/L - 0.7 g/L). The physico-chemical analysis is conducted while respecting its organoleptic properties to get at the end an energy drink with a high nutritional intake.

2. Materials and Methods

2.1. Material study

The process water was removed aseptically using 3 sterile 225 ml glass bottles of the storage tank. The valve was well cleaned with a disinfectant (alcohol 90°) and then rinsed. After letting the water run for a few minutes, the bottles were filled and closed.

• 30 g of grape concentrate was taken aseptically under hygienic conditions and introduced into 3 sterile vials.

• 30 g of sugar were collected aseptically in ultra-hygienic conditions and introduced into 3 sterile vials.

• Spirulina powder (Vita Spirulina) N ° lot:. 011,413,790 (Spirulina available is in the form of original high-quality Morocco powder), this powder is kept away from moisture. It is rich in bioactive components and nutritional substances. This gives it a high potential for use, especially in the diet, as a supplement rich in protein. The nutritional properties of Spirulina make it a food source that deserves special attention for its development.

• Semi-finished product (before pasteurization grape juice).

• Finished Product: these are the bottles cardboard Tetra Pak capacity of 1l which are the subject of our study.

2.2. Sampling

The study focused on five different productions, five products were the subject of our study namely grape concentrate, water, the semi-finished product (before pasteurization), the finished product, and the finished product enriched with spirulina. We have prepared a juice "grape nectar" enriched using different doses of spirulina. For this drink, we prepared four samples with different doses of spirulina. The composition of each sample is as follows:

-specimen 1: contains spirulina 0.1g in 1 liter of grape nectar.

-Specimen 2: contains spirulina 0.3 g in 1 liter of grape nectar.

-Specimen 3: contains spirulina 0.5g in 1 liter of grape nectar.

-Specimen 4: contains spirulina 0.7g in 1 liter of grape nectar.

-Specimen Preparation

To prepare these 4 samples, are mixed:

• 1 liter of juice "grape nectar"

• Spirulina at the different doses

- Mix the 500ml grape juice with the selected spirulina dose in a beaker and shake well for 5mins with an agitator.
- Reverse this mixture into the remaining 500ml of grape juice, and mix it again for a few minutes with a stirrer.

2.3. Methods of physicochemical analysis

2.3.1. Physicochemical Analyses performed on water

The physico-chemical analyzes are carried out on two types of water : water boiler, and that of the process.

- pH Principle: The pH was measured directly using a pH meter. Result: Direct reading of pH on the pH meter.

-Title gauging water (TH) : according to the standard(Bezerra et al., 2008).

Principle : Total hardness or title of a hydrometric water corresponds to the sum of concentrations of metal cations. In most cases it is mostly due to ions Ca^{++} and Mg^{++} (METHOD complexometrically). If the resulting solution is blue, so TH = 0. If the resulting solution is violet, proceed to the titration Solution Ethyl Diamine TetraAcetic acid (E.D.T.A) 0.02 N to turn blue.

$$\mathbf{TH = 1000.\ C.\ V1/V2}$$

The total concentration of Ca^{++} and Mg^{++} expressed in mmol/ l

C : Concentration mol / l of the solution of 0.02N ETDE,

V1 : Volume in ml of the solution E.D.T.A,

V2 : Volume in ml of the sample (100 ml),

Conversion : 0.1 mmol / l = 1 °F

$$\mathbf{TH\ (°\ F) = V1}$$

-Full Title and alkalinity alkalinity water TA and TAC [4][8] (AFNOR., 1986), principle An alkaline water is estimated by the acidimetry carbonates of HCO_3

- Present therein Expression of results

$$\mathbf{TA = V1.\ 5°F\ then :\ TA = V1.10°F}$$

TA is expressed in meq is converted to level French : 1meq = 5°F

V 1: volume of H_2SO_4 used for titration.

$$\mathbf{TAC= 2V.\ 5°\ F\quad so :\quad TAC=V.10°F}$$

V: volume H_2SO_4 versed in $V_1 + V_2$ solution.

Chlorides [3](NA 6917); principle Chlorides are determined by a solution of chromate in the presence of silver nitrate potassium. The reaction is indicated by the appearance of red color characteristic of AgCl.

$$\mathbf{(CL^-)\ V. = 100\ mg / l}$$

V : volume $AgNO_3$ paid.

2.3.2. Physical and chemical analyzes of the concentrate

The physicochemical analysis is first performed at dilutions to measure the degree of Brix and Titratable acidity.

Brix (refractive index) (SI 2173 / EN 12143).

Principle, The degree of Brix is measured by refractometer. The reference temperature is 20°C. Brix value in a program is given by the following formula:

Brix = lectue.4 (g/kg)

Titratable acidity according to the standard (NA 691 / EN 12147), principle, Acidity analysis by titremetric method using a known normality basis.

2.3.3. Physico-chemical analysis performed on the semi finished and finished products.

There are four parameters to analyzes : pH, acidity, Brix, and the density. There are performed only for the finished product. Density (Al-Dhabi, 2013).

Principle, Determination of the density and the corresponding temperature of the product to be inspected by direct reading.

2.3.4. Physico-chemical analysis performed on Spirulina

Water content : The standard NA / 1133/1990) (Benahmed Djilali,2012) .

a) Principle : The method consists of a practical reference steaming at atmospheric pressure, at a temperature 130-133°C under defined operating conditions, the mass loss observed is equivalent to the amount of water present in the product.

The potential of hydrogen (pH) : According to NF V05-108 standard in July 1970. The total protein content : According to the standard (Bezerra,2008**).**

The total lipid content : According to the (Codex Alimentarius ,1992), AFNOR NFV03-713 (1984).

The total carbohydrate content "G" in g per 100 g of dry product is calculated by difference:

G = 100 - (H% + CB + C%%% + L + P%)

Along With :
H : moisture content (% of dry product),
CB : ash content (% of dry product),
P : total protein content (% of dry product),
L : total lipid content (% of dry product).
Energy value : The determination of protein, fat and carbohydrates content allowed us to calculate the energy value for each drink prepared according to the formula :
Energy value in Kcal = 4 carbohydrate + 4 proteins + 9 lipids
Determination of total ash : According to Standard No. 22.97.07

2.3.5. Sensory analysis

The choice of the best formulation is based not only on the nutritional qualities but also on the organoleptic qualities which led us to perform the sensory analysis.

Sensory analysis therefore aims to describe the sensory characteristics of the products in an objective and quantifiable way according to defined criteria appearance, texture, flavor and aroma (ISO 6222, 2011)
A questionnaire was distributed to people from different backgrounds (academics, officials and employer of the manufacturing unit Vita juice).

For each sample, the tasters noted the level of overall acceptability using a structured 4-point scale. As the sensory evaluation is focused on the general acceptability, Note 1 corresponds to poor quality product on the taste level, odor and appearance and Note 4 corresponds to a good quality product.

2.3.6. Juice stability test

The stability test was conducted on the best chosen formulation in sensory analysis (F3 juice). This juice was prepared and analyzed the same day and then analyzed after incubation at 22 ° C for 4 weeks.

The main interest is to observe the evolution over time to estimate a deadline for optimal use and to study the stability of this juice and its physicochemical, organoleptic and microbiological quality.

2.3.7. Dissolution of phycocyanin

Based on its pharmacological properties, phycocyanin, is studied as an active substance, deserves attention from the point of view of release phenomena from tablets immersed in various liquid media (distilled water, HCl 0.1 N and phosphate buffer solution pH 6.8).

The dissolution test consists of placing each tablet in 500 ml of dissolution liquid in the dissolution apparatus (Figure 1) equipped with paddles operating at 50 r.p.m and the temperature of the medium is set at 37± 0.5 °C (Sriamornsak, 2007) .
Phycocyanin content is calculated at different time intervals using the colorimetric method described by Jourdan (Jourdan, 2012) which involves measuring absorbance at 615 and 652 nm.

Phycocyanin concentration was obtained by tuning equation 15:

$$\mathbf{M_{ph_t} = 1,873 * (DO_{615} - 0,474 * DO_{652}) * \frac{DIL}{C}}$$

Equation 1

DIL : Dilution factor;
M_{ph} : Initial phycocyanin concentration (%);
DO_{615}, DO_{652} : The optical density (OD) measured at wavelength 615, and 652 nm respectively (m-1) ;
The concentration of dry spirulina soaked in water around 4%.
The results obtained, percentage of dissolved phycocyanine compared to the initial quantity in the tablets are represented as a function of time in the form of a graph.

2.4. Statistical analyzes of the results

For the interpretation of the results obtained, the data were subjected to a statistical analysis:
An analysis of variance to determine the effect of the doses on the studied parameters and their interactions. The significance of the results is expressed according to their probability.
the classification of homogeneous groups was carried out by the test NEWMAN and KEULS which makes it possible to classify the values at a threshold of 5%.

A correlation analysis was performed between the physicochemical parameters of the samples.
These statistical analyzes were performed using STATISTICA software. Version 6.

3. RESULTS

3.1. Results of physico-chemical analysis

3.1.1. Process Water

Physicochemical analyzes were performed to verify the effectiveness of these treatments. Knowing that this water has a direct influence on the organoleptic quality of the product.

The results of the physicochemical analyzes of the process water are shown in table 1

Table 1. *Results of physicochemical analysis of the semi-finished product*

Analyses Specimen	TH	pH	Cl-
E1 11/05/14	5,7	7,30	50
E2 13/05/14	4,9	7,25	60
E3 15/05/14	8,5	8,46	35
E4 18/05/14	19	7,55	38
E5 21/05/14	10	7,22	40
Standards	=10	7 à 8,5	Max 40 mg/l

The results of the physicochemical analysis of the process water, showed that:

The pH of the samples varies between 5 (7.22 to 8.46), which complies with the internal standards of the Vitajus unit.

TH of the four samples (E1, E2, E3, E5) is consistent with the standard except the E4 is superior to the standard. This non-compliance of the TH is probably due to high concentrations of metal cations that are typically calcium and magnesium and resulting in the saturation of an ion exchange resin (softener). This latter also requires regeneration.

Chloride of the three samples (E1, E2) is greater than the standard. The presence of excessive chloride in water promotes corrosion (when water is not charged in limestone, it is often corrosive to metal water pipes. In each case, it may follow significant cost of repairing pipes (leaks or blockages), so it is necessary to reduce the chloride concentration because it will influence the organoleptic quality of our finished product.

3.1.2. Semi finished product

The results of physicochemical analysis carried out on the semi-finished product are shown in Table2.

Table 2. *Results of physicochemical analysis of the semi-finished product.*

Analysis Spicemen	acidity	refractive index	pH	Density
E1	3,50	15,2	2,93	1,064
E2	3,92	15,3	3,14	1,065
E3	3,78	14,9	2,78	1,062
E4	3,50	15	2,90	1,063
E5	3,38	15,4	3,02	1,059
Standards	3,22 à 4,20 g/kg	14,6 à 15,5 Bx	2,50 à 3,20	1,058 à 1,064

The results show that the five samples conform to the standard established by the Vitajuice unit. This reflects the respect of the recipe when preparing the doses of five samples, thus the product can be pasteurized and packaged.

3.2.2. *Finished product*

The results of physicochemical analyzes carried out on the finished product are summarized in Table 3.

Table 3. *Results of physico-chemical analysis of the finished product.*

Analyzes Samples	Acidity	Refractive index	pH	Density
Control	3,64 b†	15,1 a	2,74 c	1,064
0.1	3,22 a	15,3 a	2.72 a	1,065
0.3	3,36 a	14,9 c	3.09 d	1,062
0.5	3,64 b	15 b	2,80 cd	1,063
0.7	3,22 b	15,0 a	3,18 b	1,059

The statistical analysis (Newman-keuls test) showed that they are not significantly different between all parameters at the probability 0.05 propability level there is a significant effect ($p < 100$) of the dose of spirulina incorporated on the parameters studied (Acidity ,pH, Density , refractive index). The Brix degree of all the samples prepared varied between (3.22 and 3.64), however we can say that the Brix values of 5 samples meet standards required by J.O.R.A.

The four samples have almost the same pH ranging between (2.72 and 3.18) on average and that does not exceed the critical limits that are between (2.50 and 3.20). The density ranging from (1.058 to 1.059), these samples have a density according to the standard which is of (1.058 and 1.064). The results obtained for acidity meet the standards required by Vitajus company, which confirms the physico-chemical quality of the finished product.

3.2.3. Spirulina

The results of physicochemical analyzes of spirulina are shown in Table 4.

Table 4. *Physico-chemical analysis of Spirulina.*

Tests	H%	Dry	pH	Ash content %	Protein content
Spirulina	6.14	93.86	8.03	10.79±0.1	61.25±0.17
Standards	<10	>90	7-9	<10	>50

According to (AFNOR, 1986). We note compliance of all the results: pH, dry matter, moisture, ash content and protein content. For Humidity (% H) and dry matter (DM%) : Moisture is a very important factor in the physical and chemical characteristics of Spirulina. There is 6.14% in the case of Spirulina. This value is very close to that found by (Norme NF EN ISO 6222, 1999**)** for dried products (between 4-6%). Furthermore, it should be noted that our result is lower than that obtained by (NF V03-713,1984). Which are around 13.62%. In fact, the higher the humidity, the more microbial proliferation and less material is stored. In addition, these results show that Spirulina is very rich in dry matter with a rate of 93.86%, which corresponds to the standard announced by (AFNOR,1986) which is greater than 90%.

3.2.4. Sensory analysis

Stability test F3 juice

The results of sensory analysis are shown in Figure 1.

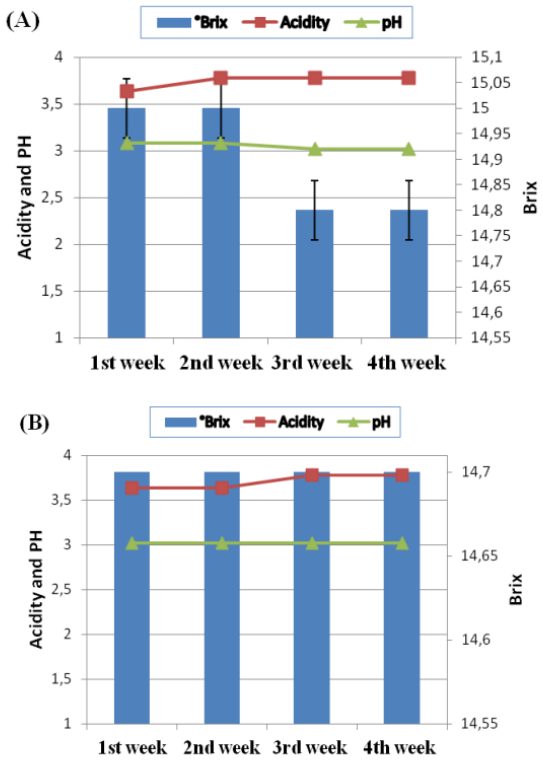

Fig. 1. *Variation of stability parameters of juice enriched at 22°C for 28 days (A) and witness juice (B)*

Standards : °B (14,4 - 15,5) Acidity (3,08 - 3,78) pH (2,50 -3,20)

The stability test results show that the values of the acidity, Brix, pH juice grape enriched with spirulina for 1 week 2nd week 3rd week and 4th week are in line with standard. Note that the enriched juice is stable, there is no change in the physicochemical criteria.

For the values obtained from the control juice (not enriched), they showed no changes occurred in the first and the second week, so a good juice physico-chemical quality.

By the fourth week we observe a decrease of the index of refraction to 14.8 but it remains at the norm, with a stability of pH values and acidity.

These analyses are accompanied by a taste test, and we note that there is no change in sensory quality (taste, aroma with excellent color characteristic grape juice). The results of physicochemical analysis of the 4th week of the control revealed that juice Brix values (14.5), pH (3.02) and acidity (3.78) are included in the standards. The results of organoleptic analyses indicate that at the 4²th week the color, the taste and the smell did not undergo any modification. This affirms the Sensory quality of our juice.

3.2.5. Microbiological analyses of the formulation F3 (0.5g)

The total germs sought are germs at 30 ° C, Total coliforms at 37 ° C, 30 ° C Yeasts and molds 30 ° C.

The formulation F3 is considered stable. The absence of microbial flora in the samples treated with steam is only a confirmation of the results of the physical and chemical analyses, in the physical aspect in particular (no bending of the bottles). The pH variation does not exceed 0.5 units. This can be attributed to the quality of raw materials, the efficiency of the heat treatment applied to grape juice and juice final before steaming and the hygienic conditions in the preparation and analysis. The results shown in Table VI revealed that it is enriched juice of a

good microbiological quality. We demonstrate by the end that spirulina has not contaminated the juice.

3.2.6. Nutritional value of the enriched juice

The nutritional value is the quality of food according to the nutrients that it contains.

This test was performed on a grape juice fortified with Spirulina (0.5g / l) to study its nutritional value.

The following report represents (Table 5).

Table 5. *The nutritional value of grape juice enriched with spirulina (0.5g/l) According Lbq*

Determinations	Unit	Control juice	Enriched juice	Methods
Sugar content(carbohydrates)	%	12.00	14.00	Bertrand
Lipid content	%	0.01	0.02	Petroleum ether extraction
Protein content	%	0.12	0.43	Kieldhal
Energetic value	Kcal/100ml	48.57	57.90	Calcul

The results of the nutritional value performed on the enriched grape juice and the control, obtained from an external laboratory (LBQ).

The results of the nutritional value of the enriched grape juice showed that the protein content increased compared to the finished product (ordinary grape juice).

The results of the energy value performed on the enriched grape juice demonstrate an increase in value compared to the energy value of the unfortified juice (Finished product). From these results, we can note that spirulina influences nutritional value with the increase in protein and therefore increasing the energy value.

3.2.7. Dissolution of phycocyanin

The dissolution process is naturally influenced by the tablets' abilities to erode and disintegrate (Benahmed Djilali et al.,2011). The release rate of phycocyanin from our tablets (Figure 2) increases significantly in distilled water compared to the other two liquids examined, this could be due to the molecular structure of the investigated substance and its high affinity to water (hydrophilic) (Hirata, 2000), but the low release rate (17% in 90 minutes) of phycocyanin is an important factor in terms of its therapeutic properties since a low dose of phycocyanin is more effective as an anti-inflammatory substance (Degbey, 2006).

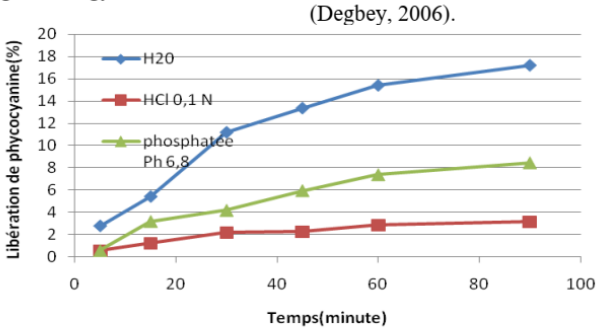

Fig. 2. *Phycocyanin release rate as a function of immersion time for different media.*

The results are in agreement with the investigations about different phycocyanin dissolution solutions (Jayant Mahadev, 2005). This author found that the highest concentration was obtained with distilled water and phosphate buffer solution at pH=6.8 while HCl 0.1 N leads to a negligible phycocyanin diffusion rate, around 2% (Benahmed Djilali et al.,2011). On the other hand, several studies have shown % release after 1 h of 30% for paracetamol (Parojcic,2007).and 12% for metronidazole (Limmatvapirat, 2008), These results make the tablets more interesting for melting in the mouth (neutral pH)

CONCLUSION

The results of analysis of the nutritional value of enriched juice confirmed a significant increase in protein (31%) and carbohydrates (2%), relative to the unenriched product, we also note that the energy value has considerably increased compared to unenriched finished product; it allows us to judge our grape juice as hyper protein. The stability test consists of studying the evolution of grape juice enriched over time in order to estimate a deadline for optimal use, we noticed that the grape juice is not fermented for 4

weeks at room temperature 22°C. Our results led us to choose a grape juice stable and hyper protein with a good bacteriological, physicochemical and organoleptic quality.

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