Journal of Fundamental and Applied Sciences

ISSN 1112-9867

Available online at http://www.jfas.info

Special Issue

In celebrating the 10th anniversary of JFAS

FORMULATION OF AN ORAL EMULSION FOR THE DELIVERY OF ACTIVE SUBSTANCES CONTAINED IN *Atriplex halimus* L. LEAVES

M. Slamani[1], N. Zaouadi[1,2*], D. Gharbi[1], Y. Nait Bachir[1], A. Hadj ziane-Zafour[1]

[1]Chemical Engineering Laboratory, Process Engineering Department, Faculty of Technology, University of Saad Dahlab-Blida 1, soumaa 09000 Blida, Algeria

[2]Agri-food department, Natural and Life Sciences faculty, university of Blida 1, soumaa, 09000 Blida, Algeria

Received: 11 September 2019/ Accepted: 02 October 2019 / Published online: 01 January 2020

ABSTRACT

This work focuses on the formulation of an oral emulsion for the vectorization of active substances contained in the leaf extract of A. halimus. In order to optimize the formulation parameters of the double emulsion, a complete factorial design of 9 experiments was used. Microscopic observation, which indicates a structure, composed of spherical droplets with well-delimited edges dispersed in the continuous phase. The size of the laser particles indicates average lipid droplet diameters of about 10 µm. Zeta potential values are greater than -30 mV, indicating the electrical stability of dispersed systems. The rheological study showed that viscosity is influenced by the amount of xanthan gum, oil and gum arabic. Storage stability at different temperatures showed that some formulations are more stable than others.

Keywords: *Atriplex halimus*; Creaming index; Emulsion; Rheology; Stability.

Author Correspondence, e-mail: zaouadi.nesrine@gmail.com

doi: http://dx.doi.org/10.4314/jfas.v12i1S.3

1. INTRODUCTION

Most of the plant species that grow in the world have therapeutic properties, because they contain active ingredients that act directly on the physiological processes of the human body. They are used in both traditional medicine and modern herbal medicine, as they have advantages that synthetic medicines often lack. The use of traditional medicine is widespread and of growing health and economic importance. In developing countries, the current use of traditional medicine is accessible and affordable, particularly for the world's poorest patients, given the high cost of some drugs and their unavailability on the market [1].

Medicinal plants are a very important source of antioxidants, which generally belong to the family of polyphenols, alkaloids or terpenes. They are widely sought after for their therapeutic properties acting on inflammatory or endocrine processes. *A. halimus* is a shrub widely used in traditional herbal medicine to treat several diseases such as cysts, lithiasis, urinary tract inflammation (cystitis) [2, 3-5], and diabetes, [6] found a very clear hypoglycemic effect in rats (made diabetic by alloxane) when fed with an aqueous extract of green leaves of *Atriplex halimus*, the tissue chromium of this plant would regulate blood sugar levels by activating the effect of insulin. A cutaneous and renal drainer, diuretic and depurative, it accompanies any diet that requires tissue drainage and the removal of waste and toxins [5].

Atriplex is also recommended for the treatment of malaria [4]. Berberine is a compound known for its antimicrobial and anti-inflammatory activity while piperine and its derivatives contained in the leaves of *A. halimus* are effective anticonvulsant and anti-epileptic substances [7].

According to the study previously carried out by Otal et al [8] the aerial parts of *A. halimus* could provide a valuable food for humans under particularly harsh natural conditions, as this plant is a source of dietary fiber (cellulose) at 15.4%, protein at 15.1%, vitamins (B and C) and minerals at 23% (sodium 4.41%, calcium 1.77%, potassium 2.59%, magnesium 0.32%, phosphorus 0.21%) [9]. Secondary metabolites in *Atriplex* show the presence of total phenols, saponins glycosides, alkaloids, tannins, resins, betaines and flavonoids including flavonols [10, 11-13].

This species also has many other assets for human health, including the nutritional and energy value of its tender leaves, as a food for nomads and the local steppe population. Indeed, in spring, in several regions of Algeria, the young shoots of Guettaf are consumed by humans,

preparing them like spinach [14]. Our present work is based on the extraction of bioactive molecules from *Atriplex halimus*, in order to formulate a drinkable emulsion by their vectorization. We have therefore chosen a pharmaceutical form that will aim to improve the organoleptic characteristics and correct the taste of bioactive extracts administered orally. Knowing that the extract of *Atriplex halimus* contains a mixture of hydrophilic and lipophilic molecules, we have chosen a double emulsion delivery system (W1/O/W2) allowing the simultaneous release of hydrophilic and lipophilic bioactive molecules extracted from *Atriplex halimus*.

The first aqueous phase (W1) will contain hydrophilic organic molecules and mineral salts. The oily phase (O) will contain the lipophilic organic molecules, this primary emulsion (W1/O) will be stabilized by soy lecithin as surfactant. The final double emulsion (W1/O/W2) is only the dispersion of the primary emulsion (W1/O) in the aqueous phase (W2); this double emulsion will be stabilized by two hydrocolloids which are xanthan gum and gum arabic.

2. EXPERIMENTAL

2.1. Material

The leaves of the *Atriplex halimus* were harvested in January 2017 in the Djelfa region (geographical coordinates: 34° 40′ 00″ north, 3° 15′ 00″ east) 300 km south of the capital (Algiers), Algeria. The plant has been identified in the herbarium of the ENA (The National School of Agricultural Science, Algeria). The *Atriplex halimus* was cleaned and dried in the open air, then in the oven at 45 for 48 hours. Then the plant was finely ground by a knife mill and sieved with a 100µm porosity sieve.

Soya lecithin was obtained from Bellat (Agri-food industry, Algiers, Algeria), xanthan gum was obtained from Saidal (pharmaceutical industrial group, Algiers, Algeria) and Fleurial® brand sunflower oil (Cevital, Algeria) was purchased from the supermarket in Blida, Algeria. The gum arabic was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). The water used is double-distilled water; all other products used are analytical grade.

2.2. Preparation of the extract

We mixed the ground plant and the solvent (water) at a ratio of 1/10 in a flask under agitation, at a temperature of 50°C. After 2 hours of agitation, the solution was filtered.

2.3. Emulsion formulation

Double emulsions are prepared in two steps. The first step is the formulation of the primary emulsion and the second step is the dispersion of the primary emulsion in a new aqueous phase.

First step: The extract is dispersed in water (W1), then mixed with the oil (O) containing soya lecithin, the primary emulsion (W1/O) is prepared by homogenizing the system using an Ultra-turrax agitator (IKA, Germany) for 5 minutes at 4500 RPM.

Second step: the aqueous phase (W2) is prepared by mixing xanthan gum, gum arabic (AG) and water, the prepared polymer suspensions are left under agitation for 24 hours to allow the polymers to mix well with the water. Then, the primary emulsion (W1/O) is mixed with the aqueous phase W2 using an Ultra-turrax agitator for 2 minutes at 2000 RPM.

2.4. Characterization of emulsions

2.4.1. Optical microscopy

The prepared emulsions were observed using an Optika microscope (Italy) equipped with a camera. The samples were observed at an X40 magnification.

2.4.2. Laser particle size

Droplet size was determined using a 22 MicroTec plus analysis laser particle size analyzer (Fritsch, Germany). The results are expressed in average diameter calculated automatically by the software using the Equation (1).

$$DM = \frac{\sum_{i=1}^{n} f_i \cdot x_i}{\sum_{i=1}^{n} f_i} \qquad (1)$$

Or: 'DM' is the average diameter, 'n' the number of classes dividing the sample, 'x_i' is the representative diameter and 'f_i' is the frequency. All measurements were tripled and performed at 25°C.

2.4.3. Zeta potential

First of all the formulations were diluted to 1/100 (V/V), then the solutions were introduced into the zeta potential measuring cell and then the potential is measured using a Horiba Nano-Partica SZ100 series instrument zetameter (Horiba Instruments Inc, Irvine, CA, USA). All measurements were tripled and performed at 25°C.

Rheology

The rheology study of the 9 formulations was carried out using an MCR 302 rheometer (Anton Paar, Austria). The device allowed us to obtain the different flow curves $\tau = (y)$. By applying Equation (2), it is possible to determine the two parameters "n" and "k".

$$\tau = Ky^n \qquad (2)$$

n: is an exponent to be determined (always less than 1 for rheofluidifying behaviour and more than 1 for rheo thickener), it reflects the difference with the Newtonian behaviour for which n = 1 and is called the structure index. K: is the consistency index.

Stability study

Accelerated stability was achieved by centrifugation at 1000, 2000 and 3000 RPM for 5 minutes for each formulation. The stability assessment was determined by the observation of possible creaming and/or phase separation. While the Storage Stability is done as follows, the different emulsions have been put in 12.5 cm high test tubes; the different tubes containing the emulsions are stored at room temperature (25°C) and in the oven (50°C), for 30 days. Throughout this period, the tubes are carefully checked for possible destabilization phenomena, such as sedimentation, creaming and phase separation. The creaming indices for each emulsion are calculated at days (D_0, D_{J0}, D_{20} and D_{30}) using the following formula:

$$CI = \frac{H_c}{H_O} \qquad (3)$$

Knowing that: "CI" is the creaming index; H_0 is the height of the emulsion stable at D_0 and H_c: is the height of the creaming fraction.

2.4.4. Experimental design

In order to optimize the parameters of the formulation of the double emulsion (W1/O/W2), we used a complete factorial design with 2 levels, 3 factors and 1 point in the center, 23+1 so a total of 9 experiments. The matrix of experiments is given in Table 1. The answers studied are: The creaming index at 25°C (response1) and 50°C (response 2). The results were evaluated using MODE6.0 software (version 2001, Sweden).

Table1. Characteristics and parameters of emulsions formulated from *Atriplex halimus*

N°	Characteristics			ζ potential (mV)	Mean diameter (µm)	η100	n	*K*
	W:O	(W1/O):W2	AG					
1	4:6	4:6	10%	33.09±7.54	9.43±0.13	77.96	0.437	0.971
2	2:8	4:6	10%	34.80±2.41	9.14±0.99	65.73	0.415	1.685
3	4:6	2:8	10%	38.44±5.12	8.64±0.21	93.63	0.441	1.023
4	2:8	2:8	10%	36.07±4.74	9.02±0.97	120.07	0.470	1.075
5	4:6	4:6	5%	31.52±2.14	11.51±1.05	113.92	0.531	0.750
6	2:8	4:6	5%	31.86±3.85	10.89±0.79	72.65	0.534	0.900
7	4:6	2:8	5%	32.46±6.21	10.45±0.85	65.06	0.432	0.899
8	2:8	2:8	5%	32.81±4.48	10.67±0.98	105.25	0.572	0.467
9	3:7	3:7	7.5%	34.05±5.10	9.24±0.54	86.51	0.523	1.08

3. RESULTS AND DISCUSSION

3.1. Emulsion characterization

All the prepared emulsions showed an opaque and homogeneous aspect, we observed the structure of the double emulsions which consists of spherical droplets with well defined edges dispersed in the continuous phase, these droplets contain inside other droplets of smaller size.

From Table 1, we observe that the different zeta potential values of the double emulsions are greater than -30 mV; these values indicate that the dispersed systems are electrically stable. Indeed, according to the study conducted by Hamed et al [15], zeta potential values that are greater than 30 mV or less than -30 mV allow the stabilization of dispersed systems. These negative charges of the ζ potential are due to the nature of the stabilizers used, gum arabic and gum xanthan are both negatively charged polysaccharides [15, 16]. The values of the average diameters are getting closer, and they are all around ten micrometers.

On the other hand, the rheological characteristics of the formulations are different (Table 1, Figure 1), using the power model, we have determined the values of the structure index (n) and the consistency index (K) which are the coefficients that govern this model. After the

development of rheological models, we were able to determine the viscosities at a shear rate of 100 S^{-1} (ɳ100).

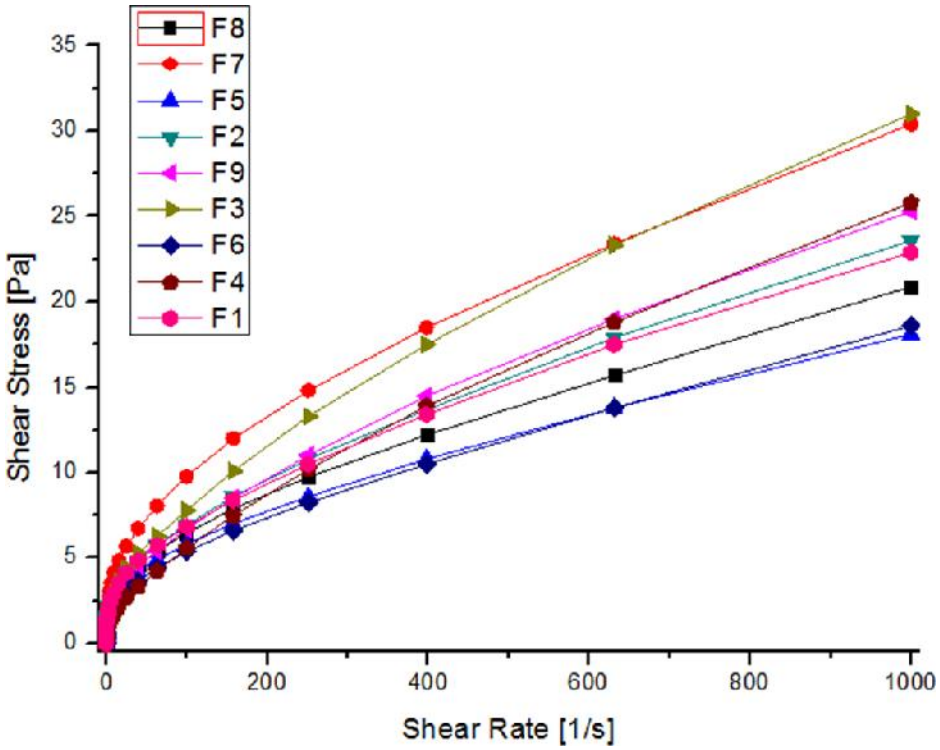

Fig.1. Emulsion flow curves

The results obtained showed that the viscosities are influenced by the quantity of the viscosifying agent contained in phase W2 (xanthan gum), the quantity of oil representing the organic phase (O) and the quantity of gum arabic.

Emulsion stability study

The accelerated centrifugal stability study showed that all formulations are stable after 5 min of centrifugation at 1000, 2000 and 3000 RPM. On the other hand, the study of storage stability at different temperatures showed that some formulations are more stable than others and that the optimum formulation is the F3 formulation.

Figure 2 shows the evolution of the creaming index (CI) for formulations during storage at 25°C and 50°C, the CI decreases at 50°C more rapidly compared to 25°C, this decrease is due to the thermodynamic destabilization of the systems studied.

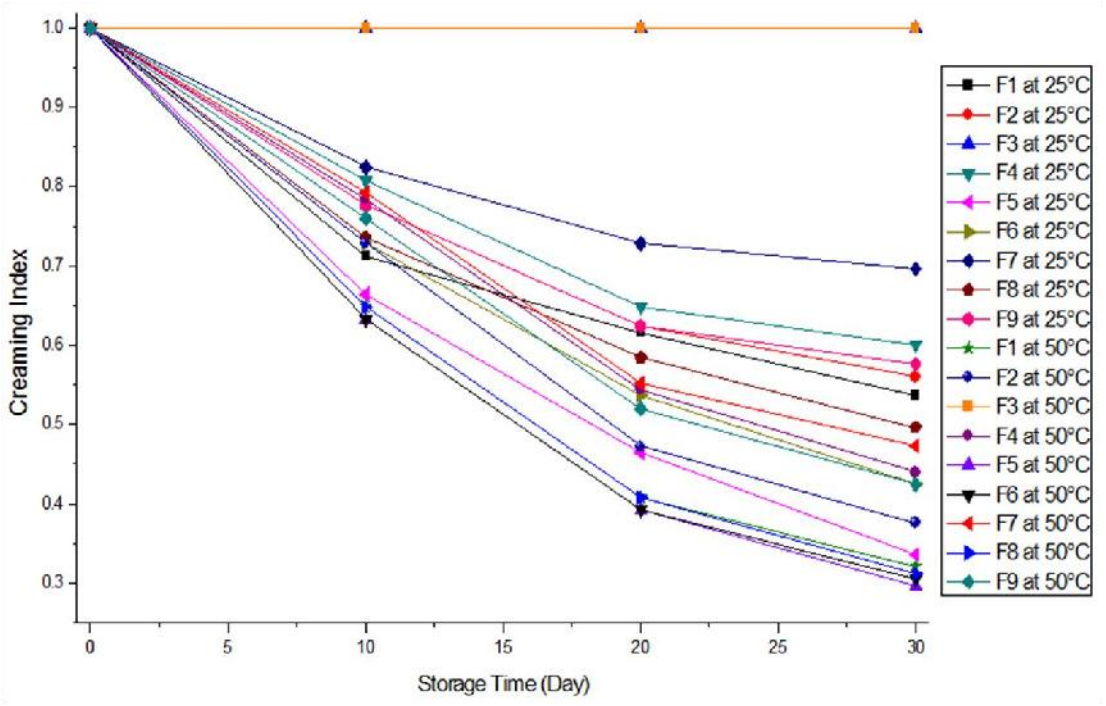

Fig.2. Evolution of creaming indices for formulations during storage at 25°C and 50°C

3.2. Effects of factors and their interactions on creaming indices

The effects of the factors (ratio W1:O, ratio (W1/O):W2 and the amount of gum arabic) and their interactions on the responses studied (CI at 25°C, and CI at 50°C) are shown in Figure 3.

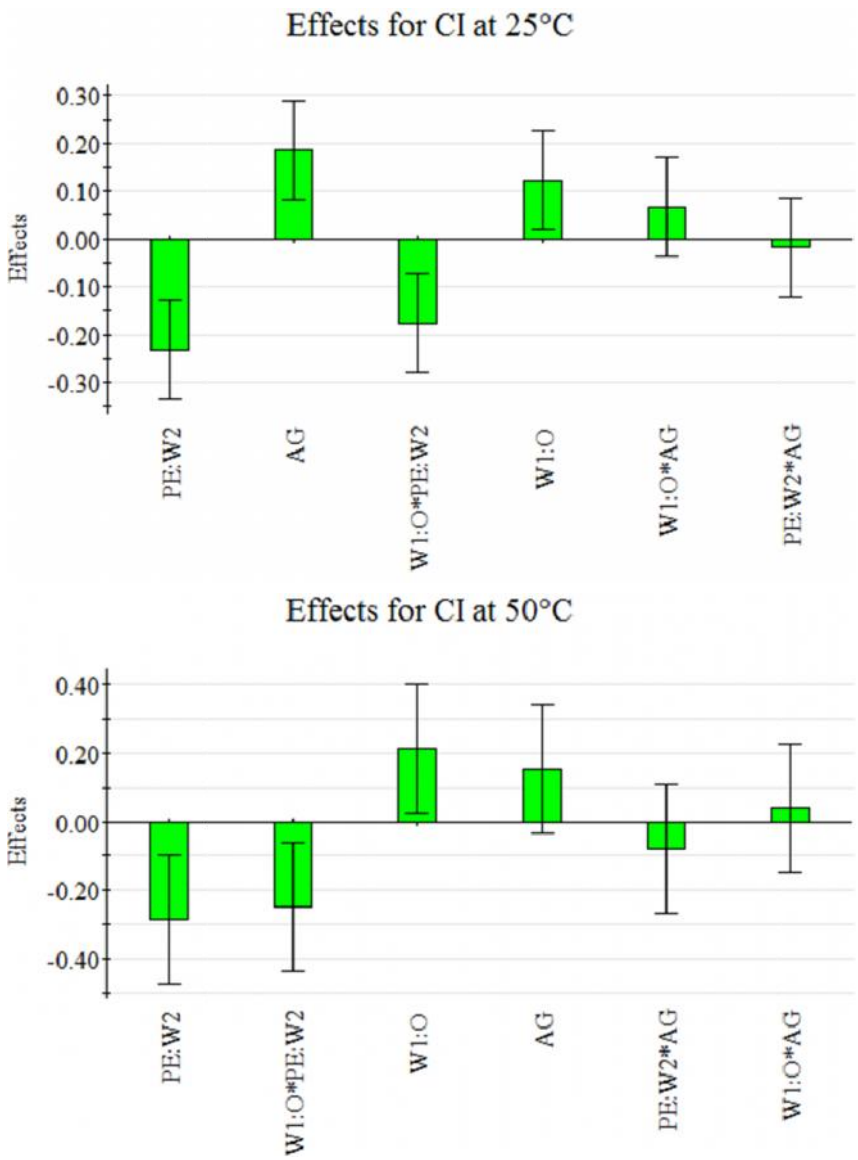

Fig.3. Effects of factors on creaming indices at 25°C and 50°C.

In general, factor 2 (the ratio (W1/O):W2) is the most influential factor on all responses, compared to factor 1 (the ratio W1: O), factor 3 (the amount of gum arabic) and the interactions between the three factors. Factor 2 (the ratio (W1/O):W2) has a negative influence on both responses (the creaming index at 25°C and 50°C), while factor 1 (the ratio W1:O), and factor 3 (the amount of gum arabic) have a positive influence. The interactions between factors 1 and 2 (the ratio W1:O, the ratio (W1/O):W2), and factors 2 and 3 (the ratio (W1/O):W2, the amount of gum arabic) negatively influence CI 25°C and CI 50°C, while the interaction between factor 1 (the ratio W1:O) and factor 3 (the amount of gum arabic) positively influence the same responses.

3.4. Mathematical modelling and prediction of creaming indices

Figures 4 and 5 represent the response surfaces showing the predicted responses as a function of the 3 factors for the creaming index at 25°C and 50°C, respectively. CI increases in proportion to the W1:O ratio and inversely to the (W1/O):W2 ratio, while this increase is proportional to the amount of xanthan gum. Table 2 represents the mathematical models that govern the parameters studied in our process. The statistical study of the mathematical models by the ANOVA program made it possible to give values P=0.024 for the creaming index at 25°C and P= 0.047 for the creaming index at 50°C.

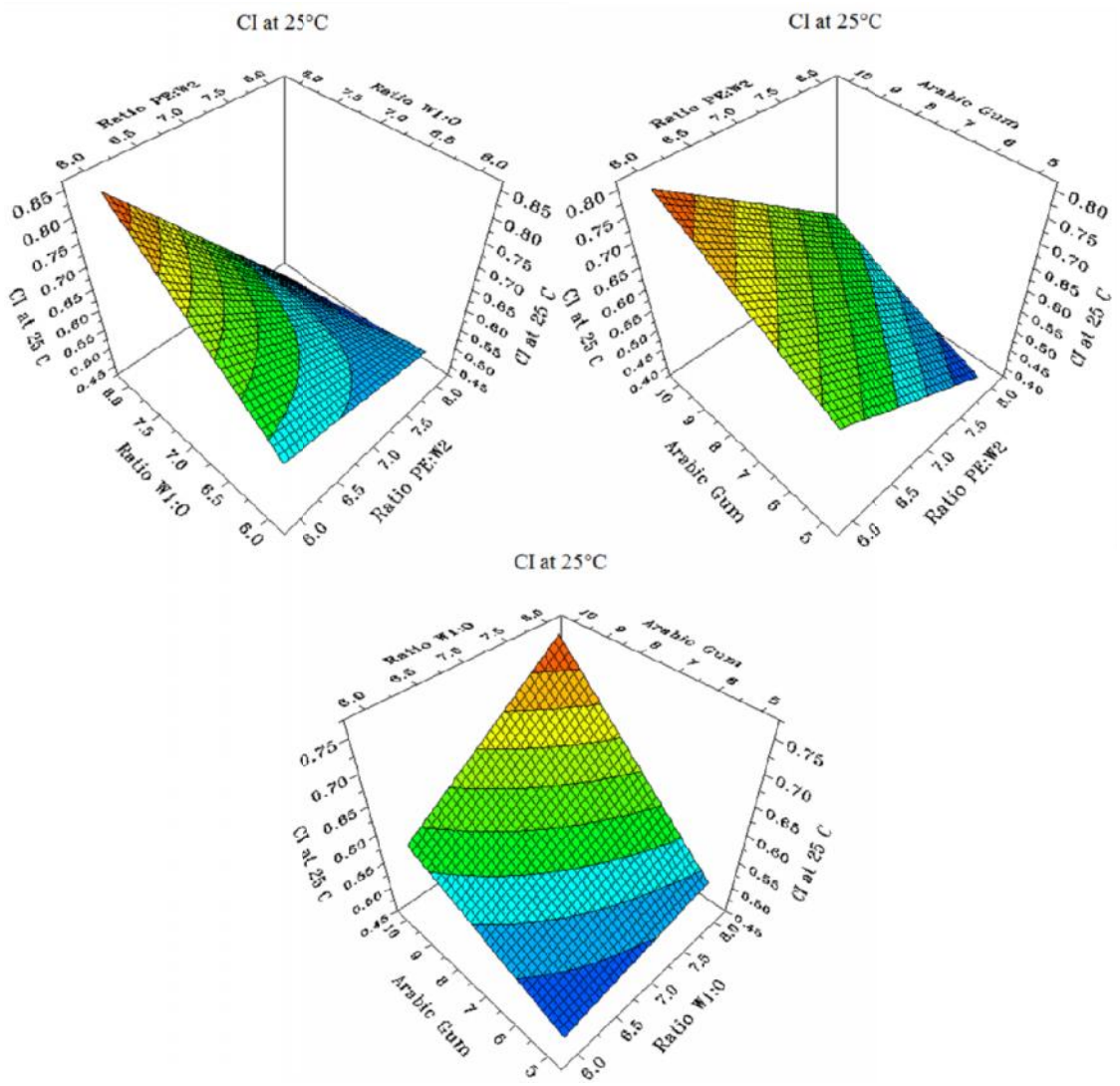

Fig.4. Response surfaces allowing the prediction of creaming indices at 25°C

Table 2. The mathematical models that govern the parameters studied in our formulation process

response	Model
CI at 25°C	CI25 = 0.580444 + 0.061 (W1:O) - 0.117 (PE:W2) + 0.093 (AG) - 0.089 (W1:O*PE:W2) + 0.033 (W1:O*AG) - 0.00899998 (PE:W2*AG).
CI at 50°C	CI50 = 0.455556 + 0.1075 (W1:O) - 0.1435 (PE:W2) + 0.0765 (AG) - 0.1235 (W1:O*PE:W2) + 0.0205 (W1:O*AG) - 0.0405 (PE:W2*AG).

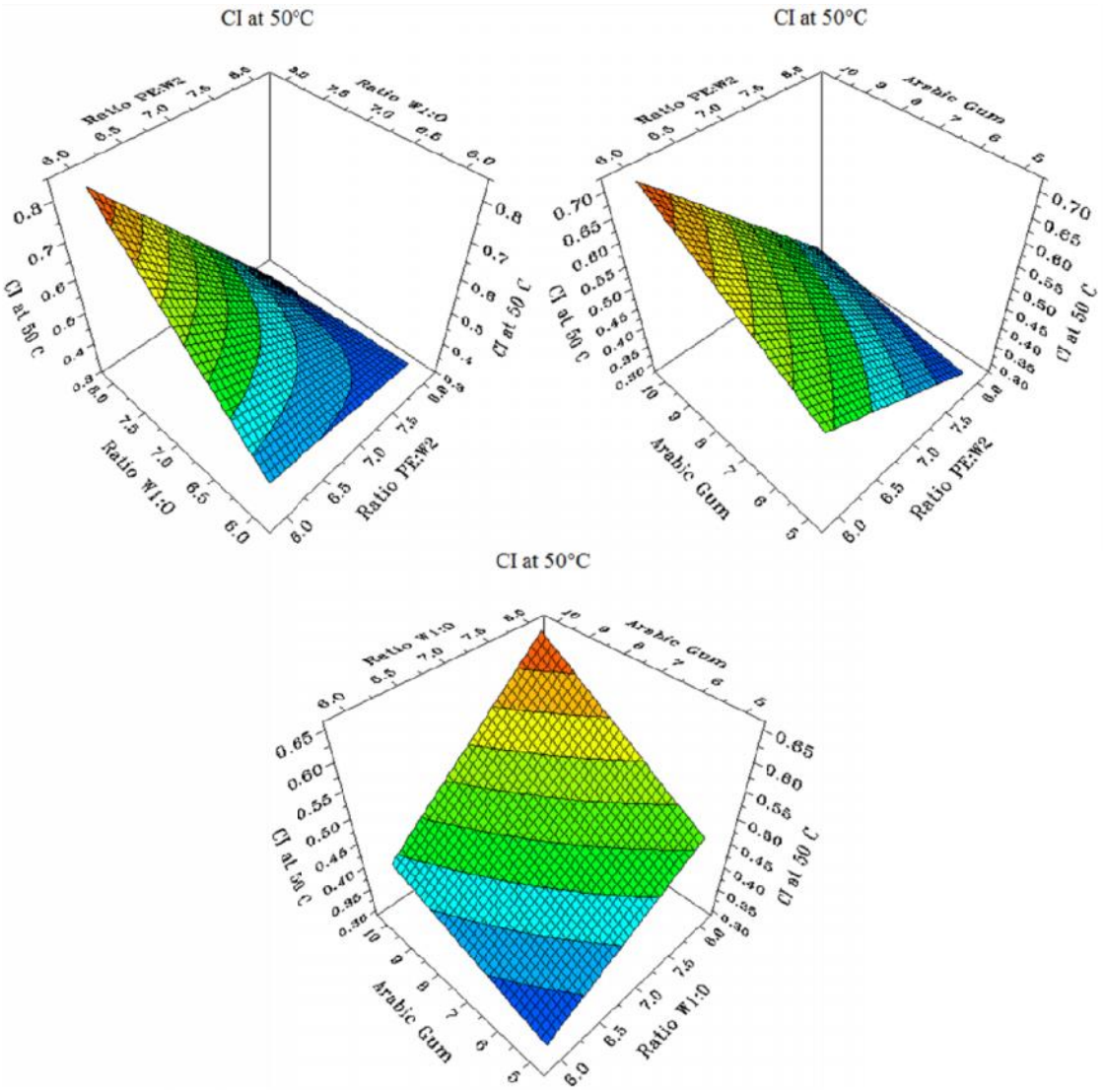

Fig.5. Response surfaces allowing the prediction of creaming indices at 50°C

3.5. Discussion of the mechanisms for stabilizing prepared double emulsion

Figure 6 shows a schematic representation of the structure of the systems developed and the different stabilization mechanisms involved. The first mechanism is based on the decrease in interfacial tension between the primary emulsion W1/O and the continuous phase W2, this decrease is ensured by gum arabic. Indeed, gum arabic has the ability to adsorb on interfacial surfaces [16]. Figure 6 shows the schematic representation of this adsorption, this phenomenon reduces the interfacial tension but also contributes to the formation of the electric cloud around the droplets called the zeta potential.

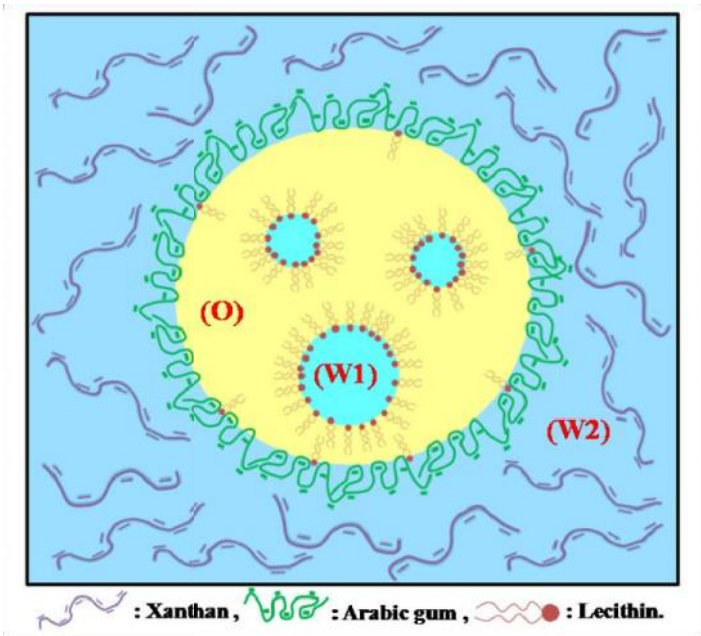

Fig.6. Schematic representation of the structure of the systems developed, showing the different stabilization mechanisms involved According to the results obtained, the stabilization of double emulsions is mainly ensured by two mechanisms

The second mechanism is based on the increase in the viscosity of the continuous phase by the viscosifying agent contained in the W2 phase. The increase in viscosity makes it possible to reduce the movement of droplets in the continuous phase and reduce their collisions, which avoids the phenomenon of coalescence of droplets, which is an irreversible destabilization of the dispersed systems and leads to phase separation [17]. This mechanism is very temperature sensitive because the viscosity of hydrocolloid suspensions is closely related to temperature. This viscosity decreases as the temperature increases [18]. This behavior clearly explains the

difference between the creaming indices at 25°C and 50°C.

4. CONCLUSION

A drinkable emulsion of type W1/O/W2 containing the bioactive molecules of *Atriplex halimus* is stabilized by a mixture of xanthan gum and gum arabic has been successfully prepared. The optimal parameters for this formulation are a W1: O ratio of 4:6, a PE: W2 (PE= W1: O) ratio of 2:8 and a quantity of gum arabic of 10%. The first objective assigned by this study was to optimize the extraction parameters of the bioactive molecules with the greatest antioxidant power.

5. ACKNOWLEDGEMENTS

We are grateful to the Institute of Applied Science and Technology (ISTA) in Blida for allowing us to do this work.

6. REFERENCES

[1] Ethics and public health; Bulletin of the World Health Organization. Volume 86, Number 8, August 2008, 577-656.

[2] Chikhi I., Allali H., Dib M. E. A., Medjdoub H., Tabti B. Asian Pacific Journal of Tropical Disease. 2014, 4(3), 181-184. DOI:10.1016%2FS2222-1808(14)60501-6

[3] Benarba B., Belabid L., Righi K., Bekkar A., Elouissi M., Khaldi, A., Hamimed A. Jrnl. of ethnopharmacology. 2015, 175, 626-637. DOI: 10.1016 / j.jep.2015.09.030

[4] Emam S. S. *Jrnl. Nat Prod*. 2011, 4, 25- 41. DOI : 10.3923/rjmp.2016.10.18

[5] Beloued A. Plantes médicinales d'Algérie. In office des Publications Universitaires, Algers , 2001, 284 p.

[6] AL-ACHI A. Herbs that affect blood glucose levels. Women's Health in Primary Care., 2005, 8, 325-330.

[7] Pei Y. Q. A . *Epilepsia*. 1983, 24(2), 177-182.DOI: 10.1111/j.1528-1157.1983.tb04877.x

[8] Otal J., Orengo J., Quiles A., Hevia M. L., Fuentes F. Small ruminant research. 2010, 91(2), 208- 214. DOI: 10.1016 / j.smallrumres.2010.03.016

[9] Boussaid M., Ben Fadhel N., Zaouali Y., Ben Salah A., Abdelkefi A. *Cahiers Options Méditerranéennes*. 2004, 62, 55-59. DOI : 2002/10/29-2002/11/01

[10] Bylka W., Stobiecki M., Franski R. *Acta Physiologiae Plantarum*. 2001, 23 (3), 285-290. DOI: 10.1007/s11738-001-0035-8

[11] Bylka W. Un nouveau diglycoside de flavonol acylé d'*Atriplex lttoralis* . Acta Phys Plant. 2004., 26 (4), 393 – 398.

[12] Abd El-Rahman H. H, Mohamed M. I, Gehad A. E. A, Awadallah I. M. Ameliorating the anti-nutritional factors effect in *Atriplex halimus* on sheep and goats by ensiling or polyethylene glycol supplementation. Inter. Jrnl. of Agri and Bio, 2006, 8, 766 –769.

[13] Benhammou N., Bekkara F A., Panovska T K. *Comptes Rendus Chimie*. 2009, 12, 1259-1266. DOI: 10.1016 / j.crci.2009.02.004

[14] Franclet A, Le Houérou H. N. The Atriplex in Tunisia and in North Africa. FAO, Rome, Italie, Document No. b0020, 1971 , p 249.

[15] Hamed M, Chin Ping T, Nazimah S.A. H, Salmah Y. Effect of Arabic gum, xanthan gum and orange oil contents on ζ-potential, conductivity, stability, size index and pH of orange beverage emulsion. Colloids and Surfaces A: Physicochem. Eng. Aspects., 2008, 315, 47–56.

[16] Bouyer E., Mekhloufi G., Le Potier I., De Kerdaniel T DUF., Grossiord J L., Rosilio V., Agnely F . J. Colloid Interface Sci. 2011, 354, 467-477. DOI: 10.1016 / j.jcis.2010.11.0.019

[17] Tadros T.F. Emulsion Formation, Stability, and Rheology. In Emulsion Formation and Stability. Weinheim: willey –VCH, 2013, pp. 1-10.

[18] Samil Kök M, Hill S.E, Mitchell, J.R. A comparison of the rheologicalbehaviour of the crude and refined locust bean gum preparations during thermal processing. Carbohydrate Polymers., 1999, 38, 261–265.

How to cite this article:
Slamani M, Zaouadi N, Gharbi D, Nait Bachir Y, Hadj ziane-Zafour A. and. A. Formulation of an oral emulsion for the delivery of active substances contained in *atriplex halimus* L. leaves **.** J. Fundam. Appl. Sci., 2020, *12(1S), 35-48.*