Optimization in production of yogurt enriched with phenolic compounds of carob pulp (*Ceratonia siliqua* L.) by experiment plan

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Abstract
The present work concerned the enriching of a homemade yogurt with the phenolic compounds of the carob pulp (Ceratonia siliqua L.) and the optimization of its production by following, particularly, the pH and the viscosity.
For this, a central composite plan was chosen, fixing the volume of the added aqueous carob extract (VACE) and the incubation time. These are the two influencing factors of the production of yogurt. The extraction of phenolic compounds (CP) was achieved by decoction. The CP contents and the antioxidant activity (AA) of the obtained extract were 1.71 ± 0.01 g GAE (Gallic Acid Equivalent) / 100 g DM (Dry Matter) and 79.4 ± 0.01%, respectively. From this extract, 3 VACE of 150, 300, and 450 mL were adjusted to 1 L of milk for the production of yogurt and a sample was left as a control.
The optimal conditions to produce a yogurt enriched with phenolic compounds were 446.55 mL for the VACE and 6.95 h for the incubation time. According to the obtained results there was 0.99 g GAE of phenolic compounds in a liter of processed yogurt.

Keywords: *carob pulp extract, yogurt, phenolic compounds, optimization, central composite plan.*

I. Introduction

The carob tree is ancestral with very controversial origins and its presence in the Mediterranean basin dates back to the Neolithic era (4000 years BC). Perennial evergreen tree, thermophilic, xerophilic and heliophilous, it tolerates poor soils, drought, and even salt (NaCl) concentrations up to 2.32 g/L [1, 2]. In addition to its characteristics, the carob fruit has very advantageous properties such as being rich in sugars, in fibers, in mineral salt, in phenolic compounds, and in antioxidants.

Carob has multiple and various uses and affects different sectors, moving from cattle feed to the food, cosmetic, pharmaceutical industry and even the manufacture of explosive devices [3].

Algeria is one of the first carob producers in the world (FAO, 2014). This resource deserves to be valued by using it as an ingredient in food matrices such as yogurt which is a widely consumed dairy product, appreciated by a wide range of consumers. It is an essential source of protein, especially in underprivileged areas, for children and the elderly persons, and also an important source of calcium.

Since a long time many studies and research works having carried out concerning this subject so that the manufacturing processes and the yogurt technology are perfectly mastered. In addition it has the ability to be a very good support to be incorporated into the daily diet, among food products such as fruits and cereals or added value products like honey and bacteria which enter into the regulation of digestion and intestinal transit. This ability was the motivating factor to develop one of the carob by-products which was its pulp, rich in phenolic compounds [4]. Several studies have demonstrated the beneficial physiological effects of these phenolic compounds on improving health and/or well-being as well as reducing the risk of developing certain diseases. Among others, their antioxidant properties, their anti-inflammatory, nephro-protective and gastro-protective, anti proliferative and apoptotic effects on human cancer cells, can be cited [5-10].

The idea of this work was to extract its phenolic compounds, using a method of extraction by decoction, an effective, a non expensive, an economical and a very simple method which could in no case have a negative impact on health when enriching the yogurt with this extract to give it an added value in phenolic compounds. This enrichment must be optimized so as to incorporate the maximum of phenolic compounds while

preserving the basic characteristics of the yogurt, particularly the pH and the viscosity. For this, the optimization of the manufacture of enriched yogurt was carried out by an experimental design using the central composite plan.

Therefore, the objective of this work was to study the influence of the added volume of the aqueous extract of carob pulp and the duration time of incubation on the pH and the viscosity of a yogurt enriched in phenolic compounds, expecting obtained the results to at least approach those of control yogurt (not enriched with phenolic compounds).

II. Materials and methods

A. Extraction of phenolic compounds

- *The raw material*

Carob pulp flour (CARUMA 30), with a particle size of fewer than 75 µm (98%), was used for the preparation of the extract in phenolic compounds to be incorporated into the yogurt. This carob powder was certified by ISO 22000, ISO 9001, HACCP, HALAL and BIO. The physico-chemical characteristics of this powder are mentioned in Table 1.

Table 1: Physico-chemical characteristics of CARUMA 30 Carob pulp flour.

Parameter	Values
Sugar	39.87%
Protein	4.46%
Humidity	2.29%
Fibers	14.48%
Lipids	0.58%
pH	5.15%
Ash	3.68%
Solubility in water	50-60%
Granulometry	98% <75µm

- *Method for extracting phenolic compounds from carob pulp*

The protocol for extracting phenolic compounds from the carob pulp presented in this work was established on the basis of the work reported in [11, 12] and shown in Figure 1.

An experimental model was implemented for the optimization of the extraction conditions with the application of a non expensive technology (decoction) to obtain added value molecules (antioxidants) from a by-product of carob (pulp) [11].

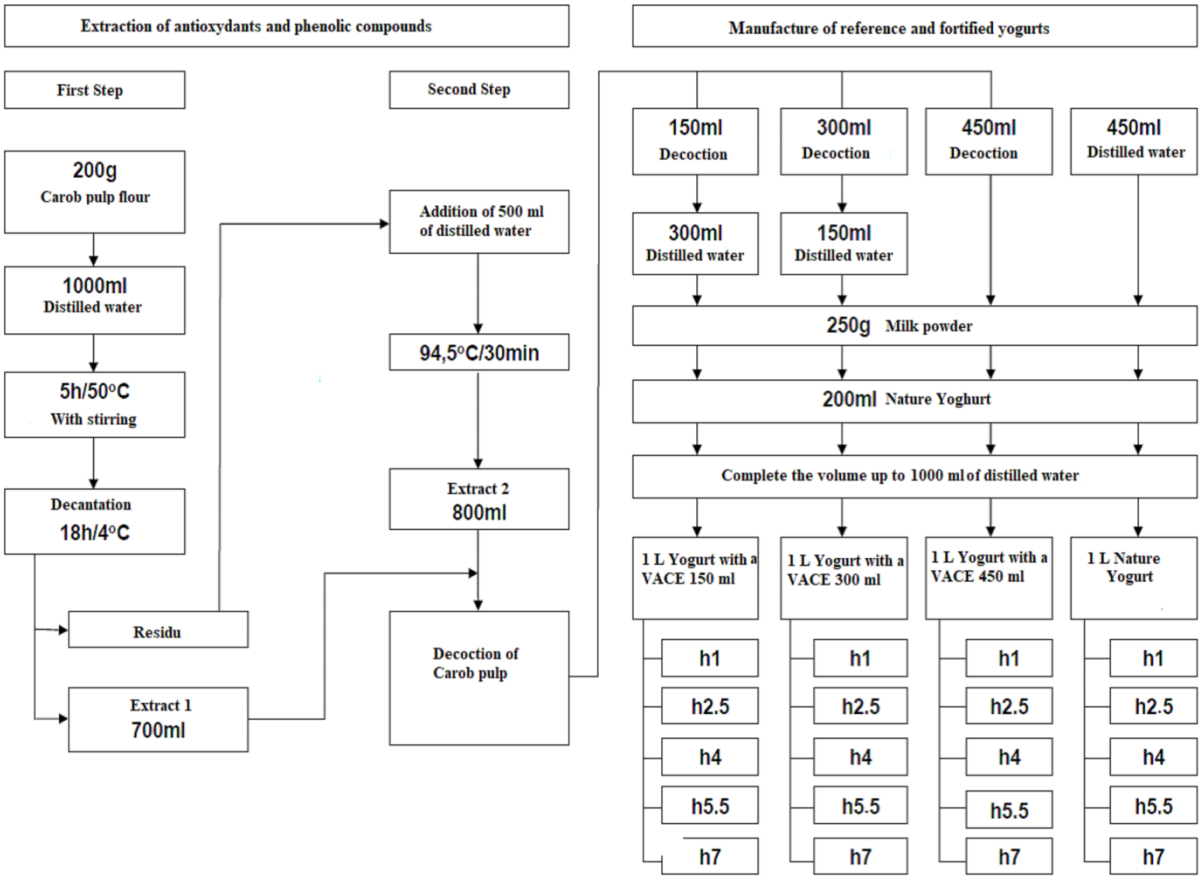

Figure 1. Extraction method of phenolic compounds from carob pulp and yoghurt manufacture proceeding

Double water-based extraction was an efficient and easy method to increase the extraction yield of phenolic compounds and to separate the carbohydrates [12]. The decoction was made in two stages. The first step was to put 200 g of CARUMA 30 flour in 1 liter of water. The mixture was placed in an agitated water bath set at 50 ° C and protected from light for 5 hours, then stored at 4°C and protected from light for 18 hours for decantation. A volume of 700 ml (the supernatant) was recovered as the first extract. The rest (the pellet) was mixed with 500 ml of water intended for the second extraction step which consisted of bringing the mixture to 94.5°C for 30 minutes (with stirring). The obtained extract was filtered through sterile cotton, and a volume of 800 mL was recovered. The extracts from the two extraction stages were mixed and incorporated into different volumes in the milk to prepare the yogurt enriched with phenolic compounds.

B. Determination of the content total phenolic compound and the antioxidant activity of the extract

- Content of total phenolic compounds

The content of total phenolic compounds was determined according to the method described in [13] using the Folin-Ciocalteu reagent which consisted of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$). These acids could be reduced by phenolic compounds, in an alkaline medium, to the blue oxide of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). The intensity of the color was proportional to the content of total phenolic compounds.

An extract volume of 100 µL was mixed with 1 mL of Folin-Ciocalteu reagent. After 3 min of incubation in the dark, 800 µL of sodium carbonate (7.5%) was added. After 30 min of incubation at room temperature, the absorbance was measured at 765 nm. A calibration line was prepared using gallic acid as standard. The content of total phenolic compounds was expressed in g equivalent to gallic acid per 100 g of dry matter (g GAE / 100g DM).

- Antioxidant activity

The anti-free radical or scavenging activity of the free radical DPPH (1, 1′-diphenyl-2-picrylhydrazyl) of the extracts of carob pulp was evaluated according to the method described in [14]. Its principle consisted in reducing the oxidized molecule of DPPH by an antioxidant by giving it a proton. The color of the solution of the DPPH radical (blue-violet) became pale yellow or colorless when completely neutralized. A volume of 100 µL of the extract was added to 1 mL of the DPPH solution (60 µM). After 30 min incubation in the dark, the absorbance of the reaction mixture was measured at 517 nm. The DPPH radical scavenging activity was calculated as follows:

$$\%DPPH = \frac{Abst - Absex}{Abst} 100 \quad (1)$$

with Abst and Absex, the absorbance of control and of extract, respectively

C. Production of control and enriched yogurts

The manufacturing protocol for yogurts enriched with phenolic compounds by different volumes of the aqueous carob extract added (VACE) is shown in Figure 1.

Under rigorous aseptic conditions, three volumes of aqueous carob pulp extract, containing 150, 300 and 450 mL of the extract, was prepared. The first two were adjusted to 450 mL. The fourth volume was prepared at 450 mL which contained only water. Enriching milk with protein was an essential step for having a better texture yogurt. Currently, it was more common to fortify yogurt by adding milk powder. For this, Gloria Nutrifort milk powder was used in the preparation of yogurt. Each liter of yogurt contained 250 g of milk powder to reach the two-thirds ratio.

The production steps were as follows: for the control yogurt, 450 mL of the water, 250 g of milk powder and 200 mL of nature yogurt (Soummam, source of ferments) were mixed in 1500 mL beaker placed on a hot plate with a magnetic stirring set at 40°C. The volume was increased to 1000 mL with water, and then the mixture was divided into 5 aliquots of 200 mL. For yogurts enriched with phenolic compounds, the protocol was the same, but the volume of 450 mL of water was substituted for 450 mL of VACE. However, the 150 mL and 300 mL VACE were adjusted to 450 mL with water and added to the mixture instead of 450 mL of water. The 4 preparations were placed in an oven regulated at 46°C.

D. pH and viscosity measurement

- pH measurement

The principle consisted in measuring the potential difference between a measurement electrode and a reference electrode joined in a system of combined electrodes. The pH was measured using a pH meter, calibrated with two buffer solutions at pH 4.7 and

10. After adjusting the temperature to 20 ± 0.2°C, the pH meter electrode was immersed in 10 g of yogurt [15]. The pH value is displayed directly on the pH meter.

-*Viscosity measurement*

The manipulation aimed to measure the viscosity (ŋ) of the yogurt product. A ball of radius R dropped at the top into a test tube filled with a product with zero initial speed. The journey time was thus measured. The content of the yogurt was homogenized in order to eliminate the presence of any serum present on the surface. Each yogurt was gently mixed three times from bottom to top using a spatula before the contents were poured into the test tube. Three repetitions of the analysis were performed.

The temperature was maintained at 20 ± 0.2°C. A volume of 80 mL of yogurt was put into a 100 mL graduated cylinder. Two marks (1 and 2) were traced on the test piece thus designating the distance (l) which was measured. Once the radius ball (R) was dropped into the product, the displacement time between the two distances is measured by a stopwatch. The viscosity is expressed by the following expression:

$$ŋ = 2/9gR^2(\int \text{ball} - \int \text{liquid})t/l \quad (2)$$

ŋ: viscosity of liquid in Pa .s (Poiseuille);
g: Gravitation constant in g (9.82m / s^2);
∫ball: Density of the ball in kg/m^3;
∫liquid: Density of the liquid in kg/m^3;
t: Time taken by the ball to travel the height l in seconds;
R: Radius of the ball in meters;
l: Distance between the two marks in meters.

E. Statistical study and experimental planning

The obtained results were analyzed using the statistical software, the JMP 13.1 Pro. To obtain maximum information with minimum experience while optimizing the production of yogurt enriched with phenolic compounds, a central composite plan (complete factorial plan) was chosen in this study.

The choice of the segment to be operated was made between 4 hours (minimum value, coded value -1) and 7 hours (maximum value, coded value +1). The optimization of the volume of the added carob extract (VACE) was performed between 150 mL (-1) and 450 mL (+1). To obtain maximum information with a minimum of experiments, a central composite plan was adopted for determining precisely the best viscosity of yogurt enriched with phenolic compounds at the desired pH. This experimental plan consisted of taking into consideration the central points of the factors studied, whether for time, 5.5h (coded value 0), or for the VACE which was 300 mL (coded value 0). Table 2 represents the values of the two factors with the coded values.

Table 2. Representation of the central composite plan with the responses enriched yogurts pH and viscosity

TRIAL	Time (h)	VACE (ml)	pH (observed)	pH (predicted)	Viscosity (observed) (Pa.s)	Viscosity (predicted) (Pa.s)
1	4 (-1)	150 (-1)	4,81	4,81	193753,463	193753,463
2	5,5 (0)	150 (-1)	4,64	4,64	585151,96	585151,96
3	7 (+1)	150 (-1)	4,46	4,47	601985,146	606550,457
4	4 (-1)	300 (0)	4,86	4,87	38861,1317	363785,249
5	5,5 (0)	300 (0)	4,71	4,7	494026,154	494026,139
6	5,5 (0)	300 (0)	4,7	4,7	494026,139	494026,139
7	5,5 (0)	300 (0)	4,7	4,7	494026,124	494026,139
8	7 (+1)	300 (0)	4,55	4,54	558229,664	524267,028
9	4 (-1)	450 (+1)	4,94	4,96	1819,11486	17668,651
10	5,5 (0)	450 (+1)	4,78	4,78	338751,933	338751,933
11	7 (1)	450 (+1)	4,59	4,59	407835,215	407835,215

III. Results and discussions

A. Content of total phenolic compounds and antioxidant activity of carob pulp extract

The content of total phenolic compounds present in the extract (decoction of 200 g of carob pulp in 1500 mL of water) was 1.71 ± 0.01g GAE / 100 g DM. This content was close to that obtained in [12]. An extract volume of 1500 mL contained the total phenolic compounds of 200 g of carob pulp used for the decoction. Knowing that the moisture content in the carob pulp flakes used was 2.29%, the 200 g of flour used contained 195.42 g DM plus 4.58 mL water. The content of phenolic compounds in extract was 1711.72 mg GAE / 100g DM, so for 195.42 g, there was 3345.04 mg GAE in 1504.58 mL of decoction us before 3345.04 mg GAE. So the content of the extract in phenolic compounds was 2.22 g GAE/L. The antioxidant activity of the extract (decoction of 200g of carob pulp flour in 1500 mL of water) was 79.4% ± 0.01. This rate was close to values reported in the literature on antioxidants from the aqueous decoction of the carob pod biomass.

B. Statistical analysis of the model of the experiment plan

A linear model of least squares adjustment was adopted the analysis of which gave the following results:

- *Effect on pH response*

A highly significant correlation was shown between the observed values and the model predicted ones as shown in Figure 2.

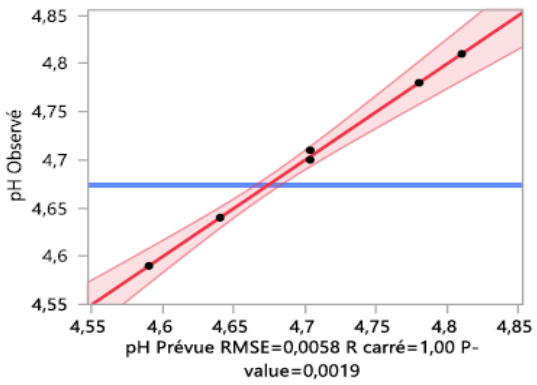

Figure 2. Comparison of the observed pH values and the predicted ones

There is also a very significant variance (p <0.01) with no lack of adjustment between the observed pH values and those predicted.

The pH value was given according to the following expression:

$$pH = 4.7033333333 + 0.18\left(\frac{Time\ (h)-5.5}{1.5}\right) + 0.07\left(\frac{\text{VACE (ml)}-300}{150}\right) + \left(\frac{Time\ (h)-5.5}{1.5}\right)\left(\left(\frac{Time\ (h)-5.5}{1.5}\right) - 0.0005 + \text{VACEml}-300150\text{VACEml}-300150-0.0066666667 + \text{VACEml}-300150 Time\ h-5.51.5-0.0005 \quad (3)$$

The estimation of the coded coefficients in Table 3 with respect to the pH response shows the simultaneous factors influence on the pH. Time influence was highly significant (p <0.001) and that of VACE was very significant (p <0.01), contrary to quadratic or crossed effects.

Table 3: Estimation of coded coefficients

Term	Coded Coefficients	Standard Error	t ratio	Prob. >|t|
Constant	4.70333	0.003333	1411.00	<.0001*
Time (h)	-0.18	0.005774	-31.18	0.0010*
VACE (ml)	0.07	0.004082	17.15	0.0034*
Time^2 (h)	-0.005	0.007071	-0.71	0.5528
VACE^2	0.0066	0.00527	1.26	0.3333
Time* VACE	-0.005	0.005774	-0.87	0.4778

The response surface of Figure 3 shows an inclination and an elevation on the time and VACE sides, respectively. This induced an inverse effect of time on the pH, the more time increased, the more the pH decreased. Whereas for VACE, the more it increased, the more the pH also increased. Therefore, there was no quadratic effect of each factor and no interaction effect between time and VACE which explained the obtained plane surface.

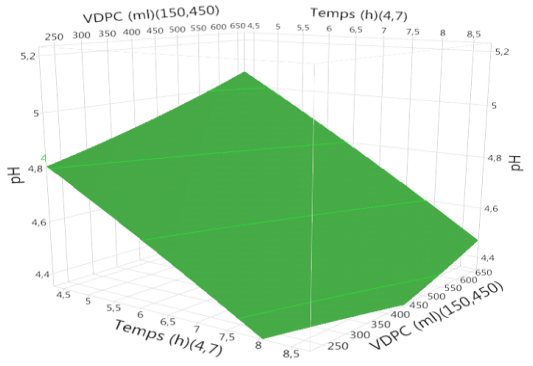

Figure 3. Response area of pH as a function of time and VECA

A similar observation was noted during the fermentation of enriched yogurt where the shortest time for the yogurt to reach a pH of 4.6 was 3 h for nature yogurt, followed by 3.5 h for yogurts supplemented with phenolic compounds and antioxidants. The more the enrichment in phenolic compounds and in antioxidants increased, the more the time of acidification also increased. The slow decrease in the pH of yogurt when it enriched a plant extract rich in phenolic compounds and antioxidants suggests an increase in the buffering capacity of yogurt, which resisted to changes in pH despite the accumulation of organic acid, but also, when the milk content of the milk contained less lactose, there would be less fermentation, therefore less lactic acid.

- *Effects on viscosity response*

A correlation between the observed viscosity values and those predicted by the model was very highly significant (p <0.0001) as it is clearly shown in Figure 4.

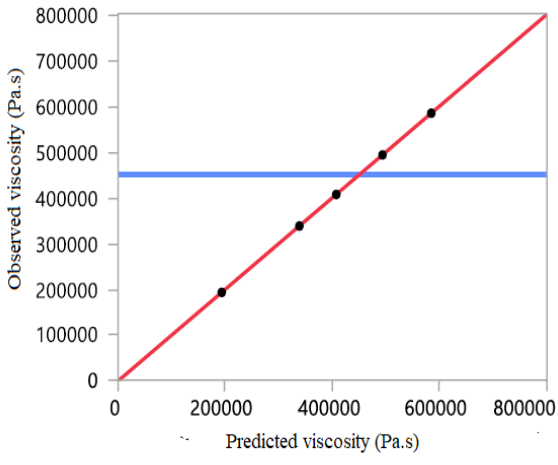

Figure 4. Comparison of observed and predicted viscosity values

As shown in table 5, the obtained model was very highly significant (p <0.0001) with no lack of

adjustment between the viscosity observed and predicted values.
The viscosity value was given according to the following expression:

$$Viscosity\ (Pa.s) = 49026.13873 + 230240.8895\left(\frac{Time\ (h)-5.5}{1.5}\right) - 123200.0135\left(\frac{\text{VACE (ml)}-300}{150}\right) + \left(\frac{Time\ (h)-5.5}{1.5}\right)\left(\left(\frac{Time\ (h)-5.5}{1.5}\right) - 187282.1922\right) + \left(\frac{\text{VACE (ml)}-300}{150}\right)\left(\left(\frac{\text{VACE (ml)}-300}{150}\right) - 232074.066666667 + \text{VACEml} - 300150\,Time\ h - 5.51.5 - 261125.048 \quad (4)$$

Table 4 shows the estimated coefficients with respect to the viscosity response. It can be seen that

Table 4: Estimation of coded coefficients

Term	Coded Coefficients	Standard Error	t ratio	Prob. >|t|
Constant	494026.17	0.008427	58626400	<.0001*
Time (h)	230240.89	0.014595	15774846	<.0001*
VECA (ml)	-123200	0.017876	-11937364	<.0001*
Time^2	-187282.7	0.013324	-10476944	<.0001*
VECA^2	-32074.19	0.014595	-2407293	<.0001*
VECA * Time	26125.048	0.005774	1789945.3	<.0001*

Time and VACE influenced simultaneously (p <0.001) the viscosity, positively and negatively, respectively and with negative and highly significant quadratic effects (p <0.001) for both factors, in addition to the effect of the interaction of the two factors studied which was also highly significant and positive (p <0.001).The response surface (Figure 5) shows us a curved shape, in the middle of the time axis; it is the quadratic effect of time. The surface declined on the viscosity side compared to the VACE and rose on the time side, a sign of the cross effect of time and VACE.

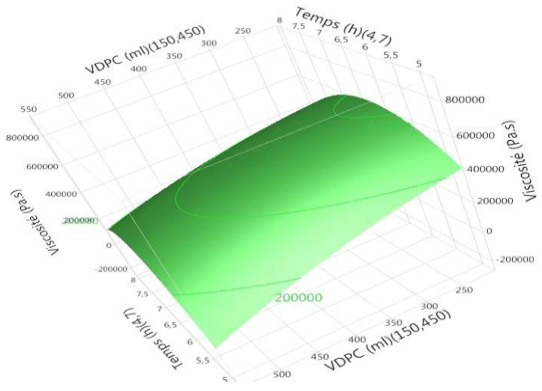

Figure 5. Viscosity versus time and VACE response surface

The model showed the simultaneous effects on pH and viscosity, quadratic and crossed on viscosity. The variances of the data were highly significant (p <0.001). No mismatch was obtained between the values observed and those predicted from the two responses studied.

This model was validated for the optimization of a yogurt product enriched with phenolic compounds.

- Summary of effects and validation of the model of the experiment plan

This model showed very highly significant effects (p <0.0000001) of the two factors studied (Time and VECA) on the obtained responses (pH and Viscosity), either separately or crosswise, with a quadratic effect of the two factors and an adjustment fault which tends towards zero (Table 5).

Table 5: Summary of the effects of the experimental design model

Source	Log Worth	P-value
Time (h)	14.396	0.0000001
VACE (ml)	14.154	0.0000001
Time * Time	14.040	0.0000001
VACE * VECA	12.763	0.0000001
VACE * Time	12.508	0.0000001

The control yogurt took 4 hours to achieve a viscosity of 415.000 Pa.s, (pH value = 4.6). This was taken as the optimal viscosity value. To reach this value, the yogurts at VACE 150 mL, 300 mL, and 450 mL had the respective durations of 4.85, 5.25, and 7.05 h.
The same phenomenon was observed in [16-17], confirming that yogurts enriched with phenolic compounds and antioxidants had significantly lower viscosity values than those of non enriched control yogurt. According to [18], the cause of these observations was probably due to the slow acidification of supplemented yogurts which negatively affected the speed of formation and stabilization of the yogurt gel.
In [19], it was also reported that when yogurt samples were fermented by different acidifying strains, low post-acidification and higher viscosity were observed in yogurt samples fortified with phenolic compounds and antioxidants compared to control yogurt. To have a clearer view of these results, Figure 5 gives the recorded viscosity for each yogurt at pH 4.6.
The viscosity compared to the VECA at the optimal pH (pH = 4.6) followed a particular shape, increasing from zero (the control), to reach its maximum at a volume of 150 mL, then it dropped at 450 mL to reach a viscosity very close to that of the control.
Many scientific research works are tried to define this phenomenon without really succeeding due to the multitude of involved factors and their

complexity. Just only a polyphenol-protein interaction model proposed in the literature in 1996 fitted perfectly with the obtained results [20].

In case, the number of polyphenols is equal to the number of protein binding sites, this should produce the largest network, which would result in larger particles and greater viscosity of the gel formed. With excess protein over polyphenols, each polyphenol molecule should be able to link two protein molecules, but it would be unlikely that all of these proteins could be linked to others. This would mainly give a weak mesh, smaller aggregates, and less viscosity. With excess polyphenols over protein, all protein binding sites would be occupied, but the likelihood of bridging would be low since each free polyphenol end would be unlikely to find a free binding site on a molecule of protein. This, too, would result in small aggregates with a weak mesh and less viscosity.

C. Forecast responses

The optimization parameters for pH and viscosity in the forecast profiler were:

- A pH between 4.5 and 4.7 with a target of 4.6;
- A viscosity between 400,000 (Pa.s) and 430,000 (Pa.s) with a target of 415,000 (Pa.s).

The forecast expression for the viscosity is exactly the same as Equation 4 but for the pH it is as follows:

$$pH = 4.7033333333 + 0.18\left(\frac{Time\ (h)-5.5}{1.5}\right) + 0.07\left(\frac{\text{VACE (ml)}-300}{150}\right) \quad (5)$$

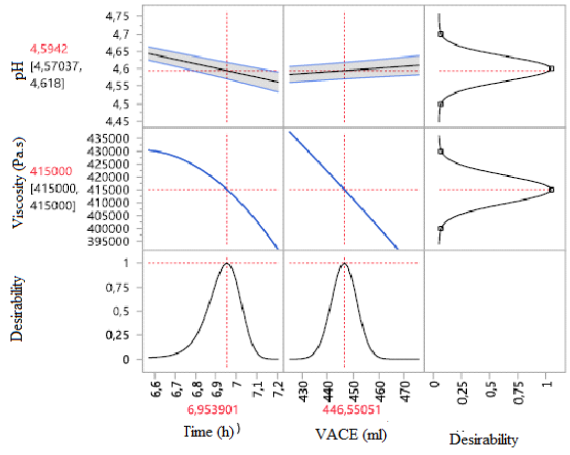

Figure 6: Forecast profiler

By maximizing the desirability of the forecast profiler, the following data were obtained to optimize our yogurt while respecting the constraints of advanced pH and viscosity: 446.55 mL of carob pulp decoction had be incorporated into our yogurt, and incubated at 46 °C for 6.95 hours to obtain a product with a pH = 4.6 ± 0.1 and a viscosity = 415000 ± 15000 (Pa.s) (Figure 6).

The results of the statistical optimization agreed perfectly with the results of the observed analyses as shown in Figure 7.

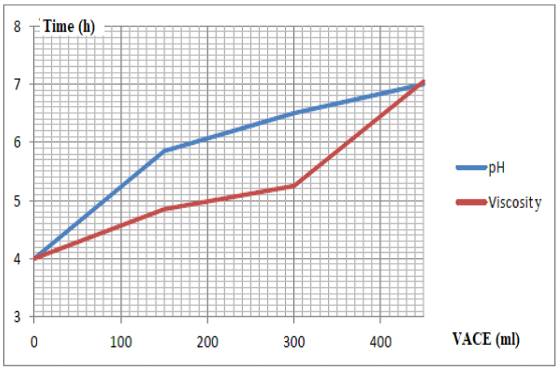

Figure 7. Optimal pH and viscosity versus time.

The forecast profiler gave an optimum added extract volume of 446.55 mL. Knowing that, 1L of decoction contains 2223.24 mg GAE, so the 446.55 mL contains 992.83 mg GAE.

So, to make a yogurt optimized in phenolic compounds, it must contain 992.83 mg GAE per liter of yogurt of these active molecules, or 446.55mL of the used carob pulp decoction, to have after 6.95 hours of incubation at 46 ° C an optimized product with a pH of 4.59 (pH = 4.6 ± 0.1) and a viscosity of 415,000 Pa.s (415,000 ± 15,000 Pa.s).

IV. Conclusion

Phenolic compounds were extracted from the carob pulp by decoction. The obtained extract was used to enrich yogurts at different volumes (150, 300 and 450 mL). An evaluation of the pH and viscosities was carried out at different incubation times (1, 2.5, 4, 5.5 and 7 hours). The obtained results were used in experimental design in order to obtain a model for optimizing the manufacturing factors of yogurt enriched with phenolic compounds from carob pulp.

The obtained results were very satisfactory and were 1.71 ± 0.01 g GAE / 100 g DM and antioxidant activity of 79.4% ± 0.01 of the extract. There was a very significant difference between the acidification times, fortified yogurts and that of the witness, or even between fortified yogurts. The rate of decrease in the pH of yogurts was proportional to the added amount of phenolic compound and the more the quantity was greater, the more the pH value decreased and vice versa. The rate of gel formation of yogurts was proportional to the added amount of

phenolic compounds. The more amounts added, the lower the rate of the gel formation, and vice versa. The viscosity followed an atypical behavior in the presence of different concentrations of phenolic compounds. At a low dose, it decreased, then by increasing the dose a little more, it increased very quickly. Once this dose increased furthermore, the viscosity decreased considerably. The statistical study of the responses allowed building a forecast model, which was used to optimize the considered factors according to the set objectives. The study carried out in this work and the optimization which resulted from it gave a very convincing result. The optimization model by the experiment plan adopted was validated and showed to be suitable to be used for the optimization of the factors studied for the manufacture of yogurt enriched with phenolic compounds of carob pulp present in the extract.

By comparing the results of the pH and the viscosity of the yogurt, optimized by the statistical model with the desirability thresholds imposed on the forecast profiler, namely a pH = 4.6 ± 0.1 and a viscosity of 415000 ± 15000 Pa.s, the best results obtained were a pH = 4.59 and a viscosity = 415000 Pa.s. This was included in the intervals of the prefixed desirability.

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References

[1] P. J. Correia, F. Gama, M. Pestana, M. A. Martins-Loução, Tolerance of young (*Ceratonia siliqua* L.) carob rootstock to NaCl. Agricultural Water Management, 97 (2010) 910-916.

[2] Melgarejo, P., Salazar, D.M.S., 2003. Fruit Growing Treaty for Arid Zones and semi-arid. Vol. 2: Algarr. 416 p.

[3] A. Haddarah, Morphological and Chemical Variability of Lebanese Carob Varieties, European Scientific Journal, l 9(18) (2013) 353-369.

[4] Y. Benchikh, H. Louaileche, B. George, A. Merlin, Changes in bioactive phytochemical content and in vitro antioxidant activity of carob (*Ceratonia siliqua* L.) as influenced by fruit ripening. Industrial Crops and Products, 60 (2014) 298–303.

[6] I. C. Theophilou, C. M. Neophytou, A. Kakas, Carob and its components in the management of gastrointestinal disorders. Journal of Hepatology & Gastroenterology, 1 (2017) 5.

[7] K. Rtibi, M. A. Jabri, S. Selmi, A. Souli, H. Sebai, J. El-Benna, M. Amri, L. Marzouki, Gastroprotective effect of carob (*Ceratonia siliqua* L.) against ethanol-induced oxidative stress in rat. BMC Complementary and Alternative Medicine, 15 (2015) 292-300.

[8] K. Rtibi, M. A. Jabri, S. Selmi, H. Sebai, J.C. Marie, M. Amri, L. Marzouki, J. El-Benna, Preventive effect of carob (*Ceratonia siliqua* L.) in Dextran sulfate sodium-induced ulcerative colitis in rat. RSC Advances, 6 (2016) 19992–20000.

[9] M. M. Ahmed, Biochemical studies on nephroprotective effect of carob (*Ceratonia siliqua* L.) growing in Egypt. Nature and Science, 8 (2010) 41-47.

[10] S. Klenow, M. Glei, New insight into the influence of carob extract and Gallic acid on Hemin induced modulation of HT29 cell growth parameters. Toxicology In Vitro, 23 (2009) 1055-1061.

[11] L. B. Roseiro, A. P. Rauter, C. S. Tavares, J. C. Roseiro, Antioxidants from aqueous decoction of carob pods biomass (*Ceretonia siliqua* L.): Optimisation using response surface methodology and phenolic profile by capillary electrophoresis. Industrial Crops and products, 44 (2013) 119-126.

[12] M. Almanasrah, L. B. Roseiro, R. Bogel-Lukasik, F. Carvalheiro, C. Brazinha, J. Crespo, M. Kallioinen, M. Mänttäri, L. C. Duarte, Selective recovery of phenolic compounds and carbohydrates from carob kibbles using water-based extraction. Industrial Crops and Products, 70 (2015) 443-450.

[13] V. L. Singleton, Joseph A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, American Journal of Enology and Viticulture, 16 (1965) 144-158.

[14] W. Brand-Williams, M. E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, Journal of Food Science and Technology, 28 (1995) 25-30.

[15] E. P. Kora. Interactions physico-chimiques et sensorielles dans le yaourt brassé aromatisé: quels impacts respectifs sur la perception de la texture et de la flaveur? Agro Paris Tech, 2004. PhD Thesis, Available at https://pastel.archives-ouvertes.fr/tel-00005733/document (Acceded Feb 5th, 2020).

[16] T. Paseephol, D. M. Small, F. Sherkat, Rheology and texture of set yogurt as affected by inulin addition. Journal of Texture Studies, 39(6) (2008) 617-634.

[17] B. N. P. Sah, T. Vasiljevic, S. Mc Kechnie, O. N. Donkor, Physicochemical, textural 558 and rheological properties of probiotic yogurt fortified with fibre-rich pineapple peel 559 powder during

refrigerated storage. LWT - Food Science and Technology, 65 (2016) 978-986.

[18] L. Jakobek, Interactions of polyphenols with carbohydrates, lipids and proteins. Food Chemistry, 175 (2015) 556-567.

[19] H. W. Xu, Q. Liu, Z. Gesudu, J. Sun, Z. Zhang, Y. Guo, Q. Zheng, J. Hou, Y. Yu, L Y.. Qing, B. Kwok, H. Zhang, Assessment of the bacterial and fungal diversity in home-made yogourts of Xinjiang, China by pyro sequencing. Journal of the Science of Food and Agriculture, 95 (2015) 2007-2015.

[20] K. J. Siebert, N. V. Troukhanova, P. Y. Lynn, Nature of Polyphenol−Protein Interactions. Journal of Agricultural and Food Chemistry, 44(1) (1996) 80-85.