OPTIMIZING THE INCORPORATION OF Aloe vera IN YACON SLICES (Smallanthus sonchifolius Poepp. & Endl.) THROUGH VACUUM IMPREGNATION USING RESPONSE SURFACE METHODOLOGY[1]

Optimización de la incorporación de aloe vera en rebanadas de yacón (Smallanthus Sonchifolius Poepp. & Endl.) mediante impregnación a vacío[2]

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ABSTRACT

The objective of this research was to optimize the parameters of the vacuum impregnation of dehydrated yacon (*Smallanthus sonchifolius Poepp. & Endl.*) with *Aloe vera*. Samples of yacon 40 mm in diameter and 5 mm in thickness and Aloe Gold Seal-Natural 200X (AGS) in aqueous solution (15 g/100 mL) were used. The impregnation process was tested by varying the pressure, [98.2 – 451.8] mbar, and time, [7.9 – 22.1] min, of treatment, with the volumetric and mass fractions of *Aloe vera* in the yacon samples reaching [0.0418 - 0.2023] m^3 solution/m^3 yacon and [21.1650 - 55.4374] mg aloe/100 g yacon, respectively. The optimization was determined from the results of the process and using response surface methodology, yielding maximum mass and volumetric fractions at 451,777 mbar and 22,0711 minutes. Therefore, it is possible to impregnate yacon with *Aloe vera* and optimize the process.

Keywords: Aloe, impregnation, optimization, response surface methodology, yacon

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RESUMEN

El objetivo de la investigación fue optimizar los parámetros de impregnación a vacío de hojuelas de yacón deshidratadas (*Smallanthus sonchifolius Poepp. & Endl.*) con aloe vera. Se utilizaron muestras de yacón de 40 mm de diámetro y 5 mm de espesor y aloe Gold Seal-Natural 200X (AGS) en solución acuosa (15 g/100 mL). El proceso de impregnación se realizó controlando la presión [98.2 – 451.8] mbar y tiempo [7.9 – 22.1] min de tratamiento; alcanzando fracciones volumétrica y másica de [0.0418 - 0.2023] m^3 solución/m^3 yacón y [21.1650 - 55.4374] mg aloe / 100 g yacón respectivamente. La optimización se realizó a partir de los resultados del proceso y mediante la metodología de superficie de respuesta, determinando que las máximas fracciones másicas y volumétricas se obtienen a 451.777 mbar y 22.0711 minutos. Por tanto; se concluye que es posible la impregnación al vacío de aloe en yacón y la optimización de este proceso.

Palabras Clave: Aloe, impregnación, optimización, superficie de respuesta, yacón.

1. INTRODUCTION

In recent years, numerous studies have shown that people who eat a diet rich in fruits and vegetables with high vitamin and antioxidant contents have a lower risk of developing diseases. This, in conjunction with an increase in the consumer awareness of healthy eating and lifestyles, has contributed to rapid growth in the functional food market [1].

A functional product that has been attracting interest is the yacon, a tuber from the northern and central Andean region of South America, due to its abundance of fructan and fructooligosaccharides (FOS), instead of starch [2]. Yacon consumption is correlated with human health benefits, such as hypoglycemic effects [3], [4], antioxidant activity [5], and potential chemoprevention against colon carcinogenesis [6]. Another product of interest is *Aloe vera*, which has a long history of use in the promotion of health, being one of the herbal remedies most frequently used throughout the world [7], [8]. *Aloe vera* gel contains mainly polysaccharides, phenolic compounds, organic acids, enzymes, vitamins, and minerals [9]. Moreover, acemannan, a partially acetylated polysaccharide found in *Aloe vera* gel, is responsible for biological activities [10], [11], [8].

In food processing, the enrichment of vegetable products with desirable compounds by different techniques, such as vacuum impregnation (VI), has been largely used to obtain a rapid penetration of solvated compounds in plant tissues together with a homogeneous concentration profile of the solutes in the final products [12]. VI has proven to be a useful method to enrich fruit and vegetable tissue with desirable solutes (e.g., firming and antioxidant agents, flavors, cryoprotectants, vitamins, minerals, and probiotics) able to stabilize and/or improve the sensory and/or the functional properties of food products [13], [14], [15], [16], [17], [18], [19]. During VI, the porous vegetable fraction with intercellular spaces is filled with an external solution to a degree that depends on various factors, including the applied process conditions (sub-atmospheric pressure level, process duration, and temperature), the osmotic pressure and viscosity of the impregnation fluid [20], the size and shape of the samples, the effective porosity (pore size and distribution), which affect the capillary pressure of fluids within the vegetable tissue and the response of the tissue to mechanical stress [21], [22], [23], [24].

Bearing in mind the new methodologies for obtaining foods with added value, and the wide range of possibilities for capitalizing on yacon, our aim is to evaluate the response to the application of VI with a solution of *Aloe vera* in order to create products that can be produced on an industrial scale, nationally and internationally. Accordingly, the aim of this article was the optimization of the incorporation of *Aloe vera* in yacon slices (*Smallanthus sonchifolius Poepp. & Endl*.) through VI using response surface methodology.

2. MATERIALS AND METHODS

2.1. Materials

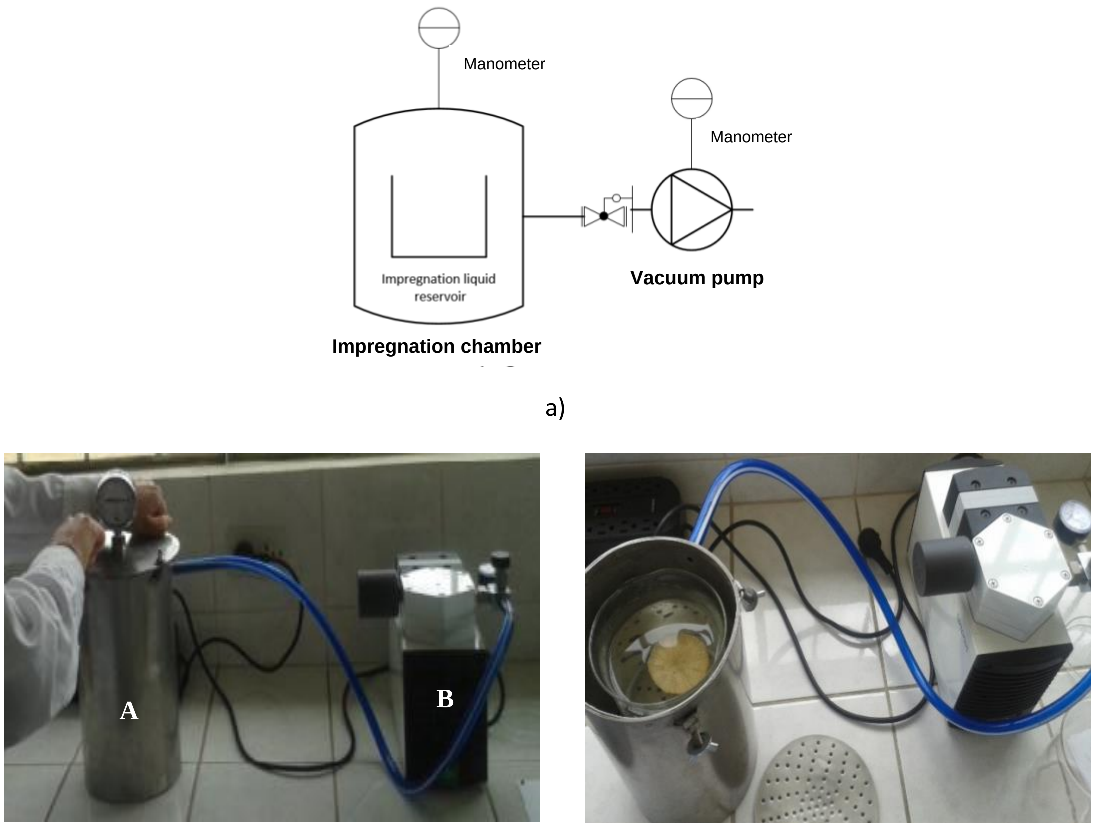
We used yacon (*Smallanthus sonchifolius Poepp. & Endl.*) acquired in a local market and a dry, pulverized *Aloe vera* extract, Aloe Gold Seal-Natural 200X (AGS) from Terry Laboratories, USA, devoid of aloin and with a total concentration of solids of 92% and a moisture level of 8%.

2.2. Chemicals and reactants

An enzyme kit for L-malic acid from Megazyme Corporation, USA, was utilized. This kit contains, among other things, glycylglycine buffer, L-glutamate, sodium azide, glutamic-oxaloacetic transaminase, L-malate dehydrogenase, L-malic acid, sulfuric acid, potassium sulfate, and boric acid.

2.3. Equipment and procedure of impregnation

Figure 1 shows the equipment used for VI. This was built based on us the works of [11], [15], [19] and [20], and it is composed of a) an impregnation chamber made of stainless steel with a sample basket and b) a vacuum pump with a vacuum pressure regulator.



a)

b) c)

Figure 1. Vacuum impregnation equipment: a) schematic view, b) frontal view, and c) yacon slice placed in the equipment

Source: Author's own elaboration

The impregnation procedure was performed according to the following steps:

- Cutting of yacon slices
- Initial immersion in solution for 15 minutes
- Placing of slices in impregnation chamber
- Application of treatment (vacuum pressure and time)
- Extraction of slices from the camera

2.4. Analysis of samples

The yacon slices and *Aloe vera* solution were analyzed before and after each treatment. The results are shown in Tables 1 and 2.

Table 1. Analysis performed on slices

Analysis	Method / equipment
Proximate analysis	Moisture AOAC 950.27
	Ash AOAC 940.26
	Protein AOAC 920.152
	Fat AOAC 948.22
Apparent density	AOAC method 942.06
Water activity	AOAC method 978.18
Color (L*a*b* space)	Konica Minolta CR400
Malic acid content	AOAC Method 993.05

Source: Author's own elaboration

Table 2. Analysis performed on solution

Analysis	Method / equipment
Density	AOAC method 945.06
Viscosity	Rotational viscometer Brookfield RVT

Source: Author's own elaboration

2.5. Evaluation of impregnation process

The impregnation process was evaluated using Equations 1 and 2, according to [22] and [26], and is described below.

$$X = \frac{M_f - M_i}{\rho_{sol}\left(\frac{M_i}{\rho_{sam}}\right)} \text{ …. Equation 1,}$$

where X is the volume fraction (m^3 dissolution/m^3 fresh yacon), M_f is the final weight (g), M_i is the initial weight, ρ_{sol} is the density of the solution (1.0034 g/cm^3), and ρ_{apm} is the sample apparent density (1.0121 g/cm^3).

$$\varepsilon_e = \frac{X}{\left(1 - \frac{P_{vacum}}{P_{atm}}\right)} \text{ … Equation 2,}$$

where ε_e is the effective porosity, X is the volume fraction, P_{vacuum} is the vacuum pressure, and P_{atm} is the atmospheric pressure.

Moreover, for each impregnated sample, the mass fraction/XHDM was determined (kg aloe/kg impregnated fruit in mg/100 g of yacon) using enzymatic analysis. The content of aloe was correlated to the malic acid content in the gel of fresh aloe.

2.6. Optimization procedure

The arrangement for the present investigation was a design based on response surface methodology using Star 2^2 with a central point, and the values used were 2 experimental factors, 3 repetitions, 6 responses, 30 runs including 1 central point per block, and 22 degrees of freedom for error.

These parameters resulted in the following operationalization levels: -1.41, -1, 0, 1, and 1.41, corresponding to times (minutes) of 7.9, 10, 15, 20, and 22.1 and pressures (mbar) of 98.2, 150, 275, 400 and 451.8, respectively, which were obtained and evaluated using the statistical software Statgraphics Centurion.

3. RESULTS AND DISCUSSION

3.1. Evolution of volume fraction

Table 3 shows the volume fraction obtained during the impregnation process with different times and vacuum pressures.

Table 3. Volume fraction (X)* of samples with different impregnation conditions

Pressure (mbar)	Time (min)	Mass (g)		X
		initial (g)	final (g)	
275.0	15.0	6.775	7.179	0.060
150.0	10.0	6.763	7.194	0.064
400.0	10.0	6.498	7.280	0.121
150.0	20.0	6.692	7.126	0.065
400.0	20.0	6.685	7.855	0.177
98.2	15.0	7.030	7.400	0.054
451.8	15.0	6.755	7.553	0.119
275.0	7.9	6.660	7.034	0.057
275.0	22.1	6.791	7.162	0.055
275.0	15.0	6.735	7.117	0.057

(*) Average of three repetitions

Source: Author's own elaboration

As seen, the volume fraction data vary according to the pressure and time used. Then, by plotting the linear regression of $(1 - P\_vacum/P\_atm)$ vs X, as in Figure 2, the effective porosity was calculated, which provides information about the index of pressure-gradient intensity [29].

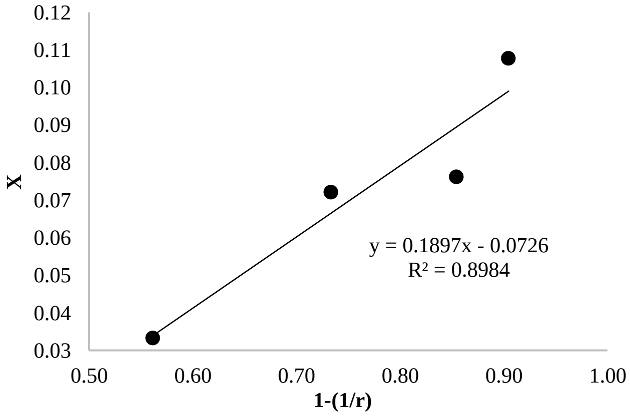


Figure 2. Volume fraction trend vs the atmospheric and vacuum pressure ratio

Source: Author's own elaboration

As seen in Figure 2, the yacon sample displayed a linear increase in its volume fraction, which makes it clear that its plant tissue is subject to low or almost no deformation. In that regard, [30] found a similar increase in samples of apple, melon, papaya, and peach and hypothesized that these operating conditions could have produced a high level of deformation (i.e., of compression) in the plant tissue, which reduced the vacuum phase available for the penetration of liquids, and the number and diameter of pores and the mechanical properties of the solid matrix, as well as the spatial distribution and characteristics of the cells and the type of fluid (liquid or gas) present in the intercellular space must be taken into consideration in order to make precise adjustments to VI treatments.

Linear regression of the experimental data shows that the volume fraction (intercept value) is an average of 0.0726, almost 0.1 m^3 solution/m^3 fresh fruit. This is similar to the value obtained in [31] of 0.19 when apple products were enriched with calcium and iron, 0.18 for *S. cerevisiae*, and 0.19 for *L. casei* in solution with apple juice. Moreover, from Equation 2 and the regression in Figure 2, the effective porosity was calculated to be 18.97%, which means that yacon can be impregnated to this percentage of its volume, similar to that the value reported in [32] of 23.6% in apple. This difference must be due to differences in the intracellular spaces in yacon, which was confirmed by [33] and [34]. These authors stated that the presence of pores in foods could be attributed to many factors since the intercellular spaces that are found in the parenchymatous tissue of fruits are not homogeneous in size.

In general, the level of impregnation is directly influenced by porosity in vegetables [29], [33]. However, while the porosity value is an indicator, it is not sufficient to characterize foods completely or to predict their behavior during VI.

3.2. Quantitative assessment of malic acid

Using as a reference the malic acid content in the gel of aloe fresco, reported by [35] as between 409–656 µmol/g dry weight or 5.48–8.80 g malic acid/100 g aloe, and Equation 1, the malic acid values (mass fraction X MHD) of the samples were calculated and are shown in Table 4.

Table 4. Quantitative assessment of malic acid (*)

Pressure (mbar)	Time (min)	X MHD (mg aloe/100 g of yacon)
275.0	15.0	22.509
150.0	10.0	30.012
400.0	10.0	31.637
150.0	20.0	26.298
400.0	20.0	32.780
98.2	15.0	24.395
451.8	15.0	54.634
275.0	7.9	32.649
275.0	22.1	27.317
275.0	15.0	33.525

(*) average of three repetitions

Source: Author's own elaboration

These results are similar to those reported by [37]. This author used 110 mbar over a period of 5 minutes, obtaining 43.0 ± 2.33 mg of vitamin C, 30.91 ± 6.91 mg of vitamin E, and 4.23 ± 0.03 mg of Zn per 100 g of fresh mushroom.

3.3. Effect of the impregnation process

During prolonged processing times, a direct relationship between high vacuum pressures and the X (Figure 3) and X MHD (Figure 4) was observed. In these Figures, the tendency for solute gains in the fruit as a function of time and vacuum pressure can be observed.

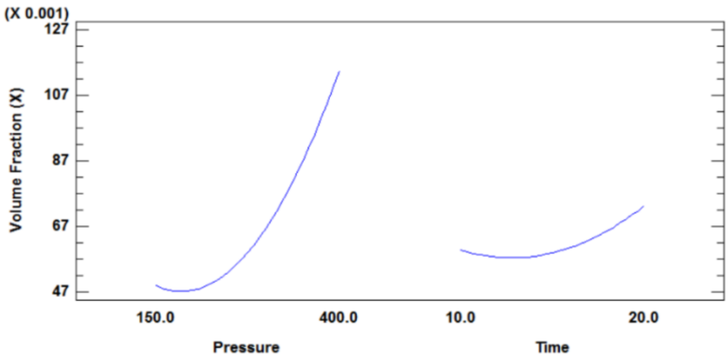


Figure 3. Diagram of the main effects on the volume fraction

Source: Author's own elaboration

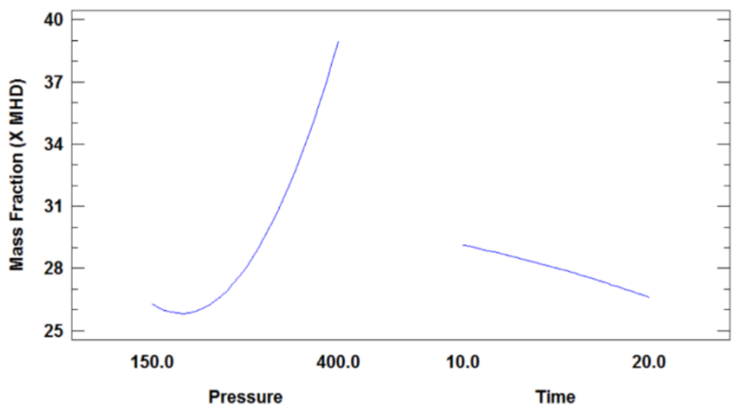


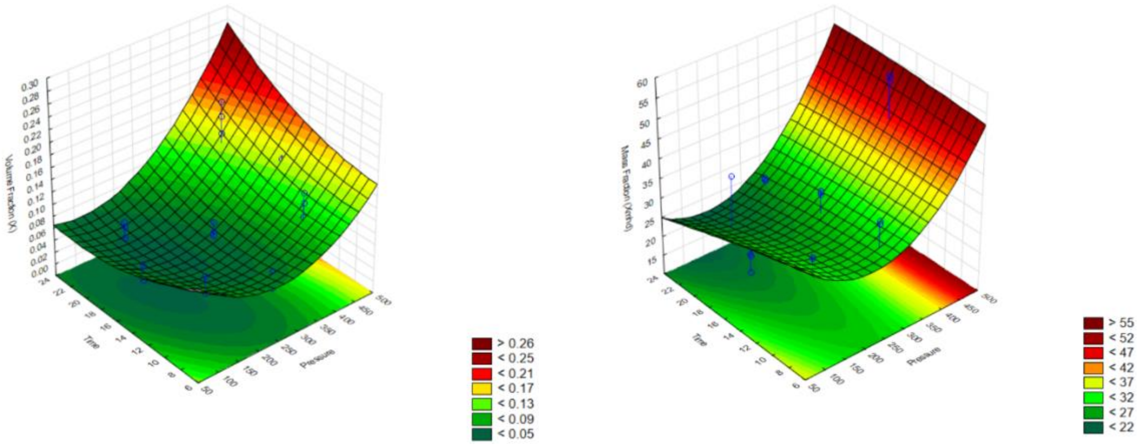
Figure 4. Diagram of the main effects on the mass fraction

Source: Author's own elaboration

The analysis of variance (ANOVA) applied to the variability of volumetric fraction (X) and of mass fraction (X MHD) for each of the effects showed a p_value = 0.0045 <0.05, meaning that these are significantly different from zero with a confidence level of 95.0%.

Among the external variables, vacuum pressure can be considered the most important, given that it represents the force that produces the pressure gradient between the vacuum phase of foods and the atmosphere surrounding the external liquid. The vacuum level is generally linked to an increase in the level of impregnation (X) because of a greater release of the plant's own liquids and gasses, together with a higher MHD and DRP (deformation-relaxation process). [23] studied the effect of vacuum pressure between 135 and 674 mbar on the volume of pores of various fruits when impregnated with an isotonic solution. For one group of these fruits, they observed a greater impregnation in line with an increase in vacuum pressure, but for the other group, the values of X increased with an increase in the vacuum level up to a maximum, after which impregnation was slightly reduced.

Figure 4 shows the estimated response surface for the effect of these relevant process variables on X (Figure 4.a) and X MHD (Figure 4.b).



a) b)

Figure 4: Response surface according to time and pressure: a) X and b) X MHD

Source: Author's own elaboration

Equations 3 and 4, which describe the estimated response surface for X and X MHD, respectively, are as follows:

$$X = 0.236 - 8.696E\text{-}4(P) - 0.014(T) + 1.454E\text{-}6(P^2) + 2.220E\text{-}5(P.T) + 3.013E\text{-}4\,(T^2)\ldots \text{ Equation 3}$$

$$X\ MHD = 46.702 - 0.140(P) - 0.596(T) + 2.949E\text{-}4(P^2) + 1.943E\text{-}3(P.T) + 6.351E\text{-}3\,(T^2)\ldots \text{ Equation 4,}$$

where T is time (min) and P is pressure (mbar), and the statistical correlation coefficients, R^2, were 76.649 and 78.393 for X and X MHD, respectively. Later, by optimizing (maximizing) the process, the highest volume and mass fraction that obtained was 46.5476, and it was obtained using a pressure of 451.777 mbar and a time of 22.071 minutes.

The vacuum period and relaxation times represent two important variables that affect the results of applying VI. When a short vacuum period is applied, the elimination of gasses from the pores is not completed, and thus the gas residues can impede penetration by the solution. As the vacuum period increases, the gasses are eliminated. An increase in the solids gain in some fruits has been observed up to a relaxation time of 30 minutes, after which the values of solids gained are roughly constant [30]. These authors put forward the hypothesis that 30 minutes is the time necessary to reach equilibrium, after which no difference in solids gain is observed. The effect that high vacuum pressures have on parenchymatous cell deformation, together with the difference in size between the molecules of the solute and water, makes diffusion of the solute through the cell membrane difficult [27].

3.4. Effect of the process on luminance (L)

In the data evaluated, three effects have a p-value less than 0.05, showing that they are significantly different from zero with a 95.0% confidence level. The most important effect is pressure, as shown in Figure 5.

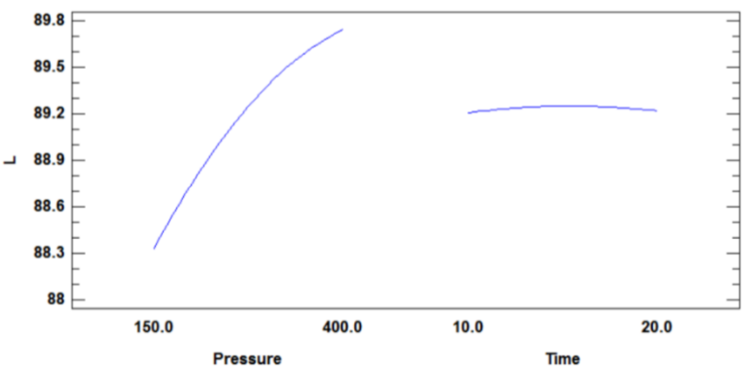


Figure 5 Graph of the main effects on L
Source: Author's own elaboration

An objective evaluation of color was carried out by changes to luminance (L), noting that the highest value obtained after the impregnation process is 89.89, which was achieved with a pressure of 98.22 mbar for a time of 7.93 minutes. According to [31], the change in color is due to the gas–fluid exchange in the process of impregnation, which homogenizes the refractive index of the sample and increases the transparency of the product.

Figure 6 presents the response surface for the L* function of time and pressure. This shows that pressure increases tend to increase transparency, which could be due to the use of *Aloe vera*, because of its chemical composition and the number of phenolic compounds that inactivate polyphenol oxidase, which is responsible for enzymatic browning in many fruits.

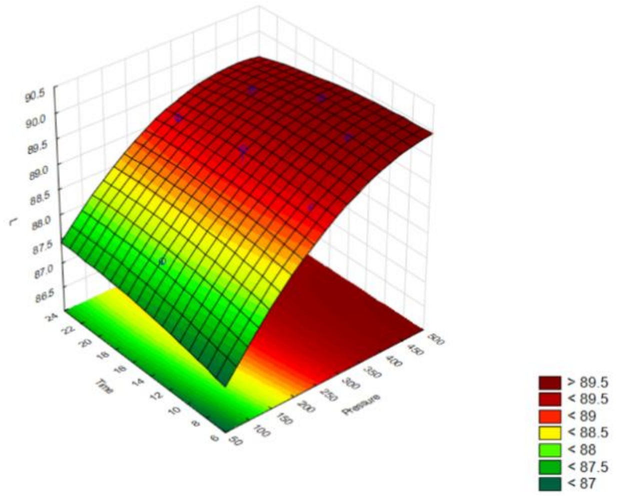


Figure 6 Response surface for the L function of time and pressure

Source: Author's own elaboration

Equation 5 describes the estimated response surface for L.

$L = 85.821 – 0.015(P) - 0.079(T) + 1.360E\text{-}5(P^2) + 1.200E\text{-}4(P.T) + 1.499E\text{-}3 (T^2)$… Equation 6,

where T is time (min) and P is pressure (mbar), and the statistical correlation coefficient, R^2, was 95.147.

3.5. Characterization of the final product

Table 4 presents the results of the physicochemical analysis of the yacon impregnated with *Aloe vera* after dehydration. The sample analyzed was the one that produced significant results in the impregnation trials with regards to the volume and mass fraction, namely, the sample subjected to a pressure of 451.8 mbar and a time of 22 minutes.

Table 5. Physicochemical analysis of the yacon impregnated with *Aloe vera* (*)

Components	Percentage
Moisture content	16.39
Dry matter	83.61
Ash	2.07
Protein	2.01
Crude fiber	2.68
Fat	0.13
NFE (carbohydrates)	76.72
pH	5.50
Acidity (exp. citric acid)	0.45
Soluble solids (°Brix)	13.8

(*) Average result of three repetitions

Source: Author's own elaboration

In the data obtained, it is worth noting the low moisture content (16.39%), which gives the product a certain degree of stability. When the flakes came out of the dryer, they had a semi-crunchy texture, which could be lost easily and quickly if they are not packaged immediately. Proximate analysis gave the following results: dry matter, 83.61%; ash, 2.07%; protein, 2.01%; crude fiber, 2.68%; fat, 0.13%; and carbohydrates, 76.72%. What stands out is the high carbohydrate content of yacon. Of the total carbohydrates, 50% to 70% are fructooligosaccharides (FOS), while the remaining carbohydrates comprise sucrose, fructose, and glucose [37]. The substitution with the *Aloe vera* solution (rich in carbohydrates) of the yacon pores typical of the gel of the parenchymatous tissue of *Aloe vera* also contributes to the carbohydrate content of the final product. According to the literature, monosaccharides and polysaccharides such as glucomannan are present in *Aloe vera*. The polysaccharides of *Aloe vera* have been proven to contribute to pharmacological activity in stimulating cell proliferation and to biological activities such as anti-inflammatory, antiviral, immunomodulatory, anti-ulcerative, disinfectant, healing, and antioxidant activities [38].

4. CONCLUSIONS

This study demonstrated that it is possible, using VI, to produce a functional product based on yacon and supplemented with aloe. The material was characterized during the process, with a volume fraction 0.0721 m^3 solution/m^3 fresh fruit and an effective porosity of 18.97%. For the established conditions in the proposed methodology, the amount of aloe incorporated into the yacon slice varied between 22.5086 and 54.6339 mg/100 g of the sample.

Moreover, using the response surface methodology (RSM), the optimization of the mass fraction and volume fraction revealed that the maximum values are obtained at a pressure of 451.777 mbar and a time of 22.0711 minutes.

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