

APPLE SLICES ENRICHED WITH *ALOE VERA* BY VACUUM IMPREGNATION

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ABSTRACT

Recently, the interest in *Aloe vera* has been increased accordingly with its content in polymannans, which show many healthy effects. The vacuum impregnation (VI) was used to enrich apple slices with *Aloe vera* gel. The effects of vacuum level, vacuum and relaxation times on the main chemical and physical attributes were described. Results showed as, applying the best operating conditions, VI allowed to introduce *Aloe vera* gel into the pores of apple tissue reaching a content of polymannan between 1 and 8 mg/100 g of fresh apples.

Keywords: fresh cut apples, polymannans, enriched fruit, aloe vera gel

1. INTRODUCTION

Aloe vera (*Aloe barbadensis* Mill.) is a perennial xerophyte belongs to the *Liliaceae* family, which consists in about 360 species. The internal fraction of the leaves is a large thin-walled parenchyma cells in which the water is held as a gel (NEWTON, 2004). The *Aloe vera*'s gel consists of water (99.5%) and solids (0.5%) such as polysaccharides, vitamins, minerals, enzymes, phenolic compounds and organic acids (ESHUN and HE, 2004; BOUDREAU and BELAND, 2006). However, a mass fraction of 60% of the total solids is constituted by polysaccharides (MCANALLEY, 1993). Recently, the interest on *Aloe vera* gel has increased on the basis of its healthy properties. Back in the past, peoples used the *Aloe vera* for its curative and therapeutic properties. Recently the intake of the gel has been proved to have positive effects in the treatment of gastrointestinal, kidney and cardiovascular diseases. Also, the gel has used to reduce the cholesterol and triglyceride levels in human blood (LIM *et al.*, 2003; GEREMIAS *et al.*, 2006). Furthermore, anti-inflammatory and antibiotic properties, as well as positive effects against several diseases, have been reported (REYNOLDS and DWECK, 1999; ESHUN and HE, 2004). Several scientific papers (HAMMAN, 2008; RODRIGUEZ *et al.*, 2010) proved the most of the health benefits may be attributed to polysaccharides (DAGNE *et al.*, 2000; NI *et al.*, 2004; HABEEB *et al.*, 2007) such as cellulose, hemicellulose, glucomannans, mannose derivatives and acetylated mannans (ROBERT and TRAVIS, 1995; FEMENIA *et al.*, 1999; LEE *et al.*, 2001). Given these considerations, food industries have been attracted from the use of *Aloe vera* as an ingredient to improve the functional properties of food products. Moreover, the FDA approved the use of *Aloe vera* gel extracted as a "dietary supplement" (RAMACHANDRA and SRINIVASA RAO, 2008). Thus, a wide number of enriched foods with *Aloe vera* such as yogurt, juice, pasta are available on the market. However, food processing could induce irreversible modifications of the polysaccharides, reducing or inactivating the health effects (FEMENIA *et al.*, 2003; ESHUN and HE, 2004; CHANG *et al.*, 2006; VEGA-GÁLVEZ *et al.*, 2011).

The vacuum impregnation (VI) is a very interesting technique that allows to introduce, dissolved or suspended substances in the void fraction (i.e. the pores) of food in a controlled manner (GRAS *et al.*, 2002). VI occurs at room temperature, avoiding the thermal degradation of nutritional and functional compounds. In the last years, several authors studied the application of VI to obtain foods enriched with structural compounds (MARTINEZ-MONZO *et al.*, 1998), probiotic microorganisms (PUENTE *et al.*, 2009), fruit juices (BETORET *et al.*, 2012; CASTAGNINI *et al.*, 2015), phenols (SCHULZE *et al.*, 2014), folic acid (MORENO *et al.*, 2016), sugars (NERI *et al.*, 2016), etc. SANZANA *et al.* (2011) evaluated the effects of VI with *Aloe vera* on the respiration rate of some vegetables (endive, cauliflower, broccoli and carrots). In some previous papers we applied the vacuum impregnation in order to accelerate the acidification of some vegetables (DEROSSO *et al.*, 2013a,b) as well as to improve the quality of thawed truffles by introducing anti-freezing proteins (DEROSSO *et al.*, 2015a).

Given these considerations, the main aim of this paper was to study the application of vacuum impregnation to improve the functional properties of apple slices by using an extract of *Aloe vera* gel. Specifically, the effects of the main process variables of VI on the level of enrichment as well as on the main physical properties of apples slices were studied using the response surface methodology.

2. MATERIALS AND METHODS

2.1. Fresh apple and Aloe vera plant

Fresh apples (cv. *Golden Delicious*) were purchased to the local market in September 2015 and stored for a maximum of 3 days at 4°C. Before treatments, the apples were equilibrated at room temperature. *Aloe vera* plant (*Aloe Barbadensis* Mill.) of three years old was supplied by Ricciotti Gardens (Foggia, Italy). The gel was prepared by cutting the fresh leaves and separating the outer green rind from the inner parenchyma.

2.2. Vacuum impregnation treatments

After washing and peeling, the apples were manually cut in slices with a thickness of 0.5 cm. The *Aloe vera* extract was prepared by dissolving 25 g of the *Aloe vera* gel in 125 mL of distilled water at room temperature under agitation at 300 g⁻¹ for 90 min. A product/solution mass ratio of 1:5 (w/w) was used for each experiment.

2.3. Experimental design

A factorial design was used to study the effects of three variables (pressure, p , vacuum time, t_v , and relaxation time, t_r) on the main quality attributes of apples (BOX and BEHNKEN, 1960). The pressure was modified between 50 and 450 mbar while vacuum times and relaxation times were studied in the ranges of 1-5 min and 5-15 min, respectively. These values were chosen on the basis of preliminary experiments performed in order to define wide ranges able to significantly increase the weight of apple slices that is a rough index of the filling of pores. More specifically, a 3^(k-p) fractional factorial design was implemented with $k = 3$ and $p = 1$ obtaining a total of 9 experimental conditions. Each VI test was repeated in triplicate by using the experimental conditions reported in Table 1.

Table 1. Experimental conditions of vacuum impregnation experiments.

Experiments	Pressure (mbar)	Vacuum time (t_2 , min)	Relaxation time (t_1 , min)
1	450	5	15
2	450	1	10
3	450	3	5
4	250	1	15
5	250	5	5
6	250	3	10
7	50	1	5
8	50	3	15
9	50	5	10

2.4. Chemical and physical analysis

Moisture (x_w) and solid (x_s) content of samples were gravimetrically measured by drying 5 g of vegetable tissue at 65°C until a constant weight (CABEZAS-SERRANO *et al.*, 2009).

2.5. Changes in porosity

Porosity values of fresh and VI samples were obtained comparing apparent (ρ_a) and real solid-liquid (ρ_r) density values. All porosity values were expressed as kg/m³. Pycnometer method was used to determine the apparent density (ρ_a) using an isotonic sucrose solution as a reference. Real (ρ_r) density and porosity fraction (ε) were estimated as reported by GRAS *et al.* (2002). The weight increase (DE) was expressed as relative differences on the basis of fresh weight.

2.6. Determination of total polymannans content

The total polymannans content was determined by using the colorimetric assay proposed from EBERENDU *et al.* (2005) with some minor changes. A volume of 400 mL of aqueous extract, 500 mL of NaOH (0.1 M) and 1 mL of Congo Red (Sigma Aldrich) dye (diluted by a factor of 500) were mixed and agitated for 2 h, then the changes in colour were analysed at 540 nm with a spectrophotometer (Perkin Elmer Lambda 25 UV/VIS). The calibration curve was performed by using a solution of β -glucan (Sigma Aldrich Inc.) in the range between 3.2 and 100 mg/L as reported by PELLIZZONI *et al.* (2012).

2.7. Statistical analysis

The effect of each independent variable on the quality indexes of apple samples was evaluated by ANOVA test with a significant level of 0.05. Also, the results were described by Pareto's charts. Moreover, 3D plots describing the changes of each dependent variable as a function of experimental conditions were obtained by fitting the experimental data with a polynomial model as reported by DEROSI *et al.*, (2015b). All statistical analyses were performed using Statistica ver. 10.0 (Statsoft Tulsa, USA).

3. RESULTS AND DISCUSSION

Table 2 shows the main physico-chemical attributes of fresh apples. Average values of moisture and solids content of *Golden delicious* were of 0.88 ± 0.01 g H₂O / g f.w. and 0.12 ± 0.01 g H₂O / g f.w., respectively. These values were in good agreement with other authors (MUJICA-PAZ *et al.*, 2003; PAES *et al.*, 2007; WU *et al.*, 2007).

Table 2. Physico-chemical properties of fresh apples.

Parameter	Value \pm SE
Moisture content (X_w) (g H ₂ O/g f.w.)	0.88 \pm 0.01
Solids content (X_s) (g/g f.w.)	0.12 \pm 0.01
Apparent density (ρ_a) (kg/m ³)	822 \pm 0.04
Real density (ρ_r) (kg/m ³)	1047 \pm 0.04
Porosity fraction (ε) (%)	21.5 \pm 0.04

Moreover, a porosity fraction of 21.4 ± 3.69 % indicates slight variability in porosity as a consequence of their biological variance, which includes the ripening degree, the environmental conditions, varieties, etc. In general, literature has reported a significant variability in porosity of apples with values between ~ 18 % and ~ 27 % (SALVATORI *et al.*,

1998; MARTINEZ-MONZÒ *et al.*, 2000; MUJICA-PAZ *et al.*, 2003; PAES *et al.*, 2007) which are in agreement with our data. As expected, polymannans were not revealed in fresh apples. With the aim to quantify the content in polymannans in aqueous extracts of *Aloe vera* the distribution function of about 40 measurements performed on extracts prepared from different part of *Aloe vera* plant, is reported in Fig. 1. A normal distribution was proved by the Shapiro-Wilk's test that exhibited a value of 0.98. The mean value was of 0.355 ± 0.078 mg/g enables to state that the most of the observation fell in the range of 0.3 and 0.4 mg/g. Also, minimum and maximum values of 0.188 mg/g and 0.546 mg/g were also observed.

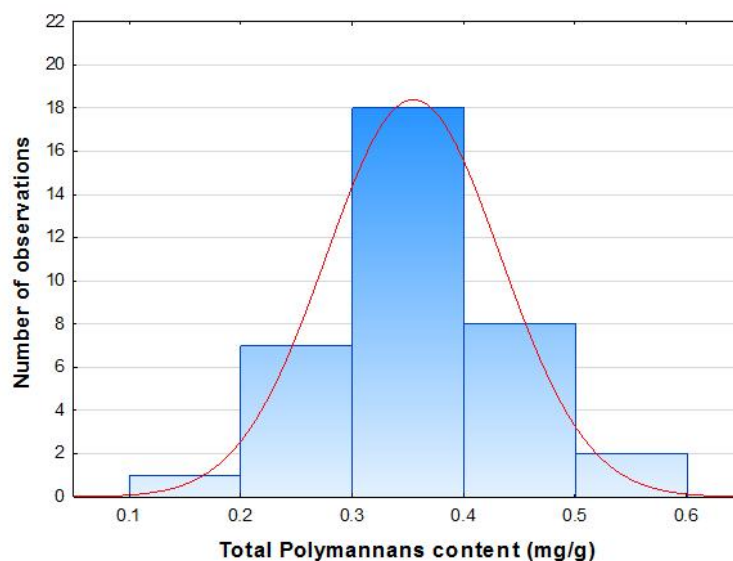


Figure 1. The normal probability function of polymannans content of the *Aloe vera* gel extracts.

The results of statistical analysis showed that the pressure value and the relaxation time linearly affected the changes in porosity of apples showing standardized effects of 6.97 and -3.53, respectively (Fig. 2a). This means that as the pressure decreased as the porosity decreased too, while the relaxation time showed an inverse relationship with the void fraction of apple tissue. Since that the driving force of VI is the difference between internal (i.e. inside the pores) and external pressures, the higher was the vacuum, the greater was the impregnation. Also, the longer was the relaxation times, the higher was the reduction in porosity, because there was more time for impregnation and relaxation phenomenon (GRAS *et al.*, 2002; MUJICA-PAZ *et al.*, 2003; NERI *et al.*, 2016). Figure 2b shows the 3D plot describing the effects of relaxation time and pressure on the porosity fraction of apples. At first, taking into account the treatment performed at the lower vacuum of 450 mbar and the minimum relaxation time of 5 min, a significant reduction in porosity from fresh apple ($\varepsilon = 21.4 \pm 3.69\%$) to 14% was observed.

Moreover, according to Pareto chart of Fig. 2a the pressure had the highest effect in the reduction of porosity fraction of the samples. According to this results porosity fraction reduced from 0.14 to $\sim 0.08\%$ when the pressures of 450 and 50 mbar were applied, respectively, with a minimum t_r of 5 min (Fig. 2b).

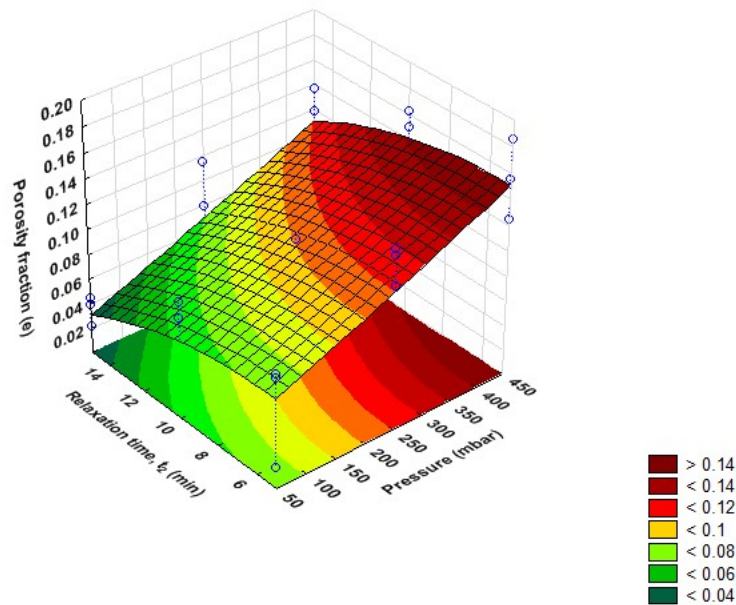
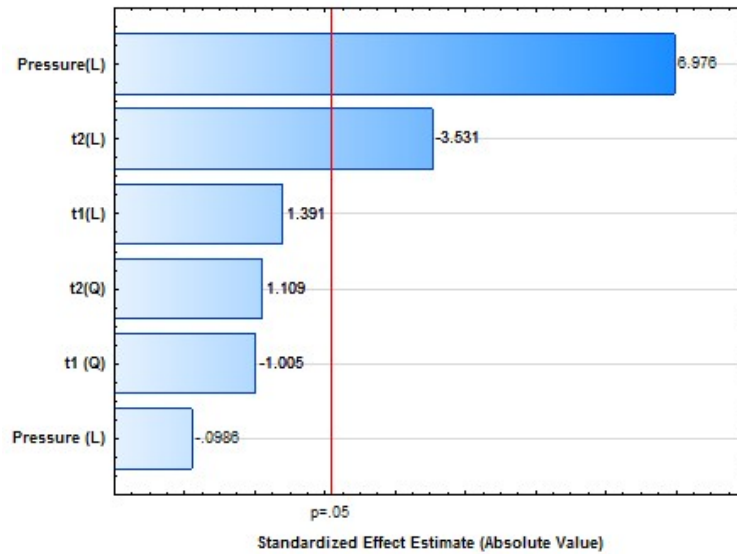


Figure 2. a) Estimated effects of the independent variables on the porosity fraction of apple slices submitted to VI treatments with *Aloe vera* gel extract. L and Q refer to the linear and no linear (quadratic) effect, respectively. b) 3D Plot describing the effects of relaxation time and pressure on the fraction porosity of apple slices submitted to VI treatments with *Aloe vera* gel extract.

On the other hand, a negligible reduction in porosity was observed by applying relaxation time from 5 to 15 min for some pressure applied. For instance, applying a pressure of 50 mbar, values of 0.08 and 0.03 were measured progressively increasing t_r . Moreover, the results stated the very high reduction in porosity fraction of apples tissue independently from the length of vacuum time; in fact, by reducing t_r a change in porosity only of 0.4% was achieved (data not shown). However, experimental data show a high variability, which cannot be underestimated. This could be the result of the variance in microstructure properties such as the porosity of fresh apples, the presence of closed pores, the size and dimension of capillaries, their tortuosity, etc. However, the decrease of the porosity fraction after VI treatment cannot assure that the impregnation by external solution

occurred. As well known, during relaxation time the impregnation and the compression of capillaries are involved, both allowing to reduce the porosity fraction of fruit. The equilibrium between the impregnation and compression level is controlled by several variables such as the viscosity of the solution, the rigidity of vegetable tissue, etc., most of which cannot be manually controlled. The Pareto chart (Fig. 3a) shows the effect of the independent variables on the weight increase of apples. The pressure linearly affected the weight increase of apple samples exhibiting a standardized effect of -3.58, while the other variables did not show any effect. The variation in weight as a function of vacuum time and pressure are shown in Fig. 3b.

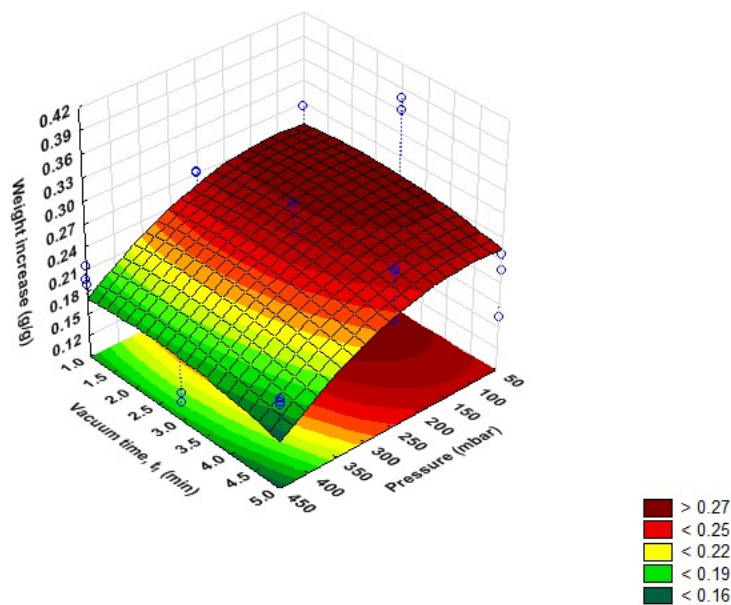
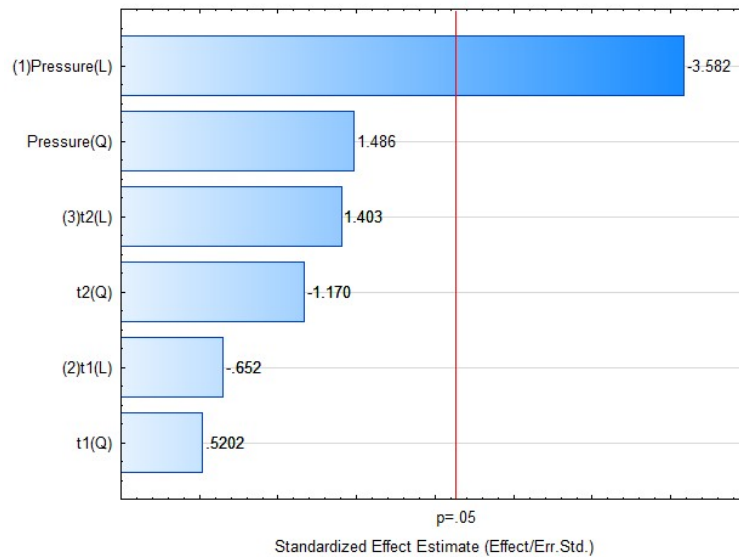


Figure 3. a) Estimated effects of independent variables on the weight increase of apples slice submitted to VI treatment with *Aloe vera* gel extracts. L and Q refer to the linear and no linear (quadratic) effect, respectively. b) 3D plot describing the effect of vacuum time and pressure on the weight increase of apple slices.

According to pareto chart, only the pressure exhibited a significant effect. Even considering the lower $t_i = 1$ min the weight of apple slices increased from ~ 0.18 to ~ 0.26 g/g by reducing the pressure from 450 to 50 mbar. That means that a significant amount of *Aloe vera* extract was introduced in the void phase of apple. On the other hand, any differences were not observed increasing the vacuum time until 5 min. Also in this case, the experimental data showed a not negligible variability that could be the result of differences in microstructure (porosity, connectivity, pore size distribution, etc.). Fig. 4a reports the standardized effects of the independent variables on polymannans content of apple slices. The pressure and the relaxation time were the most important variables affecting the enrichment of apple slices. The standardized effects of -3.15 and 2.55 showed as the pressure exhibited the greater effect on enrichment of apple samples. Fig. 4b shows the effect of the vacuum level on the polymannans content of apples.

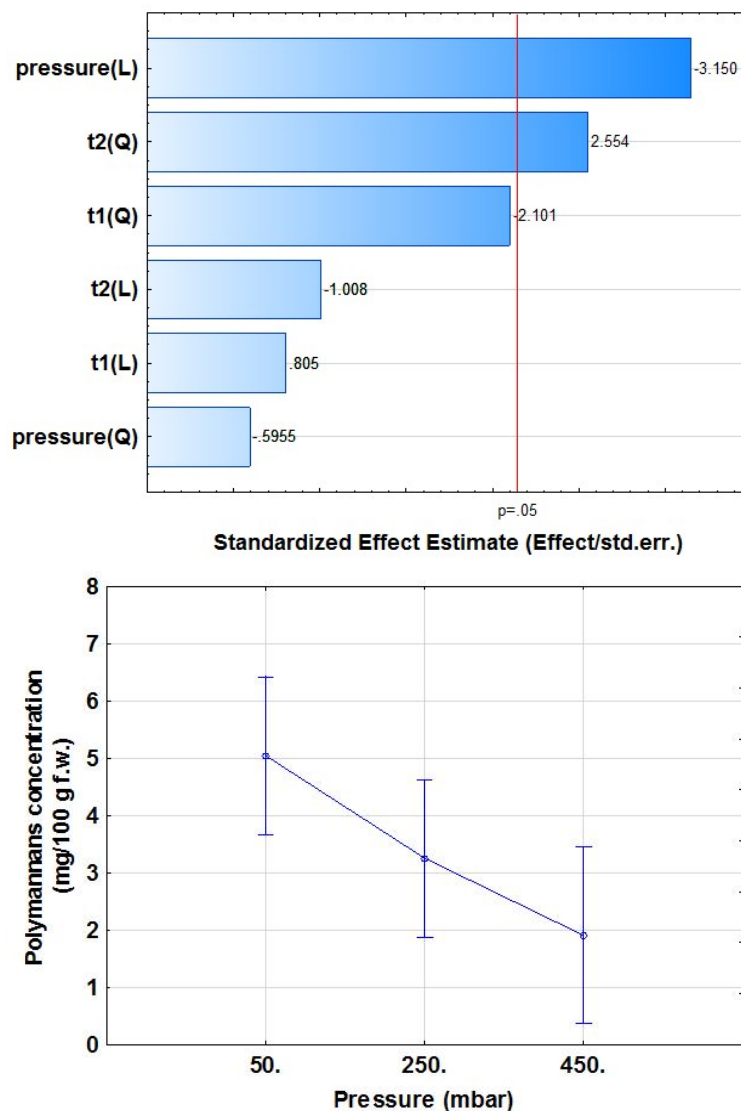


Figure 4. a) Standardized effect of the independent variables on the polymannan content of apple slice submitted to vacuum impregnation with *Aloe vera* gel extract. L and Q refer to the linear and no linear (quadratic) effect, respectively b) Changes in polymannans content of apple slices submitted to vacuum impregnation as a function of pressure.

More specifically, the figure was obtained by grouping all experimental data for the pressure values used during experiments while the other two variables, t_i and t_r , were free to change. The positive effect of the vacuum impregnation is clearly observed. By reducing the pressure from 450 to 50 mbar, the average content in polymannans increased from 2 to 5 mg/100 g f.w. This means that VI treatments may be considered a useful method to enrich apples with the bioactive compounds of *Aloe vera*. Of course, the high variability of the data (i.e. error bars) was caused by the different relaxation times as well as by the effects of the microstructure variability of fresh apple.

Fig. 5 shows the 3D plot describing the effects of pressure and relaxation time on the polymannans content of apple slices. The enrichment in polymannans was obtained for any experimental condition with values ranged between 1 and 8 mg/100 g f.w.. For any relaxation time, by increasing the vacuum level, it was possible to enrich apple slices with polymannans. An average increase of 7 mg/100 g f.w. was obtained decreasing the pressure, progressively, with a fixed relation time of 5 or 15 min. For a t_r of 10 min a peak of polymannans content was reached according to the non-linear effect reported in figure 4a. This means that a negative effect was observed for relaxation time longer than 10 min, probably because a prolonged compression damaged apple tissue favoring the release of *Aloe vera* extracts from the capillaries. On these bases, further experiments, focused on the changes in void and solid matrix phases as well as in terms of microstructure, would be necessary to improve the understanding of the behavior of apple tissues under VI treatment and to optimize the enrichment in polymannans.

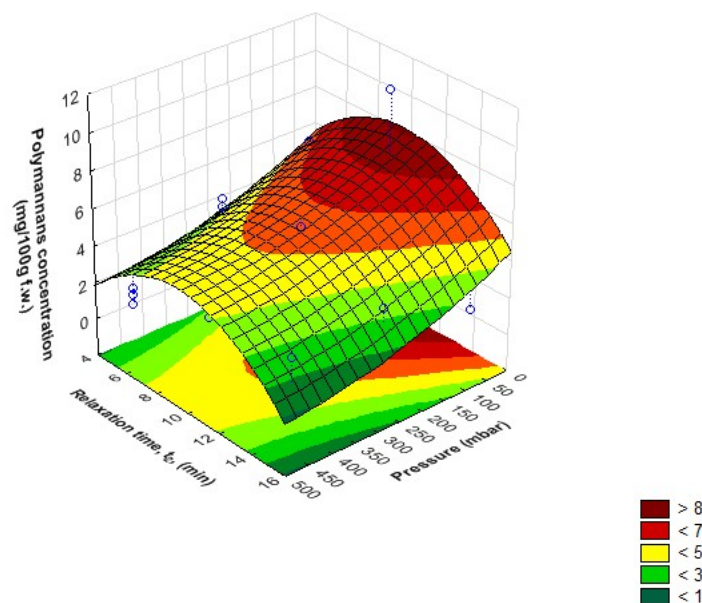


Figure 5. 3D Plot describing the effect of relaxation time and pressure on the changes in polymannans content of apples slice submitted to vacuum impregnation treatments.

4. CONCLUSIONS

By vacuum impregnation is possible to obtain fresh-cut apple slices with improved healthy properties by filling their pores with an *Aloe vera* gel extract. Pressure and relaxation time significantly affected the porosity fraction and the polymannans content of apples, while any effect was not observed modifying the vacuum time. From an initial

porosity of 21.4 % of fresh apples, values of 16% were obtained for the weakest treatments, while a value of 2% was reached for the strongest experimental conditions. The weight increase ranged between 0.16 and 0.25 g/g proved the significant impregnation of the pores by the *Aloe vera* extract. The best operative conditions were a pressure of 50 mbar and a relaxation time of 10 min by which a polymannans content of 8 mg/100 g was measured in apple slices. On the other hand a further increase in t_2 produced a decrease in polymannans content probably caused by the damage on the vegetable tissues, which reduced the capacity of the capillaries to retain the external solution.

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