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Enzymatic pretreatment for the enhancement of beetroot drying process

Abstract – The objective of this work was to evaluate the use of cellulase and pectinase as pretreatments for the drying of beetroot (*Beta vulgaris*). The experiment consisted of slices of beetroots subjected to four different treatments before the drying procedure, as follows: no wet pretreatment; wet pretreatment without enzymes; pectinase solution pretreatment; and cellulase solution pretreatment. Treatments were compared for drying rates, color change, content of betalains, and plant tissue structure. A modified Page model was used to describe the drying process. The enzymatic pretreatments did not improve the drying kinetics, although they changed the plant tissue structure. A negative influence on the drying was observed when pectinase was used; however, no effect was observed when cellulase was used. Slices treated with cellulase remained unchanged for color. Slices treated with pectinase showed significant changes of color, in comparison with the control treatments. The enzymatic pretreatments studied did not change the betalain concentrations and showed similar drying performance in the comparison with control treatments. Cellulase pretreatment is promising because it does not change the beetroot color or the betalain concentration.

Index terms: pectinase, cellulase, dehydration, enzyme, pigment.

Keli Cristina Graciola[1], Bruna Roos Costa[2], Voltaire Sant'Anna[1] ✉, Manuela Poletto Klein[3] and Kelly de Moraes[4]

[1] Universidade Estadual do Rio Grande do Sul, Campus Encantado, São José Encantado, Rua Alegrete, nº 821, CEP 95960-000 Encantado, RS, Brazil. E-mail: keli-graciola@uergs.edu.br, voltaire-santanna@uergs.edu.br

[2] Universidade Estadual do Rio Grande do Sul, Campus Cachoeira do Sul, Rua Sete de Setembro, nº 1.040, Centro, CEP 96508-010 Cachoeira do Sul, RS, Brazil. E-mail: bruna-costa@uergs.edu.br

[3] Universidade Federal de Ciências da Saúde de Porto Alegre, Departamento de Nutrição, Rua Sarmento Leite, nº 245, Bairro Centro Histórico, CEP 90050-170 Porto Alegre, RS, Brazil. E-mail: manuelap@ufcspa.edu.br

[4] Universidade Estadual do Rio Grande do Sul, Campus Cruz Alta, Rua General Andrade Neves, nº 326, Centro, CEP 98025-810 Cruz Alta, RS, Brazil. E-mail: kelly-moraes@uergs.edu.br

✉ Corresponding author

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Pré-tratamentos enzimáticos para melhorar o processo de secagem de beterraba

Resumo – O objetivo deste trabalho foi avaliar o uso de celulase e pectinase como pré-tratamentos para a secagem de beterraba (*Beta vulgaris*). O experimento consistiu de fatias de beterraba submetidas a quatro diferentes tratamentos, antes do procedimento de secagem, conforme a seguir: pré-tratamento sem água; pré-tratamento com água, sem enzimas; pré-tratamento com solução de pectinase; e pré-tratamento com solução de celulase. Os tratamentos foram comparados quanto às taxas de secagem, mudança de cor, teor de betalaínas e estrutura do tecido vegetal. Um modelo Page modificado foi usado para descrever o processo de secagem. Os pré-tratamentos enzimáticos não melhoraram a cinética de secagem, embora tenham alterado a estrutura do tecido vegetal. Observou-se influência negativa sobre a secagem, quando a pectinase foi usada; no entanto, nenhum efeito foi observado quando a celulose foi usada. As fatias tratadas com celulase permaneceram inalteradas em relação à cor. As fatias tratadas com pectinase apresentaram alterações significativas de cor, em comparação aos tratamentos-controle. Os pré-tratamentos enzimáticos estudados não alteraram as concentrações de betalaínas e apresentaram desempenho de secagem similar aos dos tratamentos-controle. O pré-tratamento com celulase é promissor, pois não altera a cor da beterraba nem a concentração de betalaína.

Termos para indexação: pectinase, celulase, desidratação, enzimas, pigmentos.

Introduction

Drying is an ancient food preservation technique (Fellows, 2018). It reduces the water activity, decreasing the enzymatic activity, microorganism growth and various physicochemical spoilage reactions (Fellows, 2018). The food industry uses drying to reduce food mass and volume, facilitating storage and reducing transport costs (Aral & Bese, 2016; Onwude et al., 2016).

Some drying processes have some disadvantages, for instance, hot air-drying negatively affects the functional, sensory, and nutritional characteristics of food (Onwude et al., 2016). New equipment, ultrasound, high pressure, and ethanol pretreatments have been adopted in the food industry to overcome the disadvantages of previous techniques (Moses et al., 2014; Monteiro et al., 2016; Rojas et al., 2020; Pandiselvam et al., 2023; Santos et al., 2023).

Enzymes have already been used in the food industry to transform raw material into main products (Souza & Kawaguti, 2021). Hydrolytic enzymes have been used to degrade cell walls of vegetables and fruits, in order to release water and intracellular compounds (Silva et al., 2019; Vivek et al., 2019; Abdullah et al., 2021; Souza & Kawaguti, 2021). An example of hydrolytic enzyme is the cellulase; it has been used to break down cellulose and hemicellulose into smaller polymers. Sometimes, cellulase is used together with pectinase (Damodaran & Parkin, 2019). Cellulase is commonly employed in the beverage industry, to improve juice extraction, enhance quality, and clarify the juice (Dal Magro et al., 2016). The release of water from plant cells has the potential for use as a food pretreatment, as it may increase the drying rate, potentially reducing the processing time and energy expenditure in drying processes.

Beetroot powder market reached a valuation of US$ 474 million worldwide, in 2023, and it is expected to grow a compound annual growth rate of 4.6% up to 2033 (Future Market Insights, 2023). Beetroot (*Beta vulgaris* L.) stands out as a source of nutrients as minerals (calcium, potassium, and sodium), dietary fiber, and bioactive compounds with antioxidant properties (Soquetta et al., 2018). Its antioxidant activity is mainly related to the high betalain content. Betalains are a class of natural pigments, including yellow-orange betaxanthins and red-violet betacyanins (Fu et al., 2020; Akan et al., 2021). However, these pigments are sensitive to heat, pH, light, and oxygen (Fu et al., 2020). Reducing the water activity of beetroots is an important strategy to enhance microbiological and biochemical stability. Thus, strategies to increase the drying rates and to reduce the negative impacts on plant nutritional contents are vital for the agro-industries.

The objective of this work was to evaluate the use of cellulase and pectinase as pretreatments for the drying of beetroot.

Materials and Methods

One kilogram of beetroots was purchased locally, in the region of Vale do Taquari, in the state of Rio Grande do Sul, Brazil. They were selected, cleaned with tap water and sliced at 1.5 mm thickness. A sample of 500 g slices were visually selected, to maintain the same standard of size and aspect. Since the slice area is variable, it was necessary to estimate the number of slices (n) for statistical purposes. The estimate was based on 1.42 g cm^3 density, 110 g fruit weight, and 6 cm slice diameter. For 500 g, the estimated number of slices was n = 84.

The sample was subjected to four pretreatments, as follows: CP, pretreatment with a solution of cellulase; PP, with a solution of pectinase; CD, a dry control control with a sample collected after cutting and not wet treated; and CW, a wet control that is second control treatment in which slices were put into water without enzymes. The experiment was conducted with three replicates. The CD treatment was used because compound leaching is critical when studying water-soluble pigments as betalains in beetroots. The CW treatment was necessary to be comparable to the enzyme solution treatments.

The cellulase enzyme used was the Celluclast 1.5 L; and the pectinase enzyme used was Pectinex Ultra SP-L (Novozymes, Araucária, PR, Brazil). The supplier LNF Latino Americana provided both enzymes. Pectinex Ultra SP-L is a blend of pectinases, hemicellulases and beta-glucanases with an activity of 3,300 PGNU per gram (Novozymes, 2022b). Celluclast 1.5 L is a cellulase solution with 700 EGU per gram of enzymatic activity (Novozymes, 2022a). Enzymes were diluted in distilled water at 45 mL L^{-1} concentration. The beetroot slices were placed in plastic bags and the enzyme solution was added at 7:3 ratio (enzyme:beetroot, v/m), as suggested by Lotfi et al. (2015) and Shavakhi et al. (2021). The packages were sealed and left to rest at room temperature (~20ºC) for 30 min. Although this

temperature is not optimum for enzymatic activity, the enzymes maintain their hydrolytic capacity at slower rates. The reason for maintaining this temperature is to simulate the conditions of small-scale businesses, where budgets are limited. After the 30-min rest period, the beetroot slices were removed from the solution and their surfaces were carefully dried with a paper towel. They were placed onto stainless steel trays (20 x 10 cm) with 10 mm holes in a single layer. A convective fixed-bed dryer with infrared heating and vertical air flow was pre-heated for 15 min at 60ºC. The dryer was constructed specifically for drying plant materials, according to the description by Sant'Anna et al. (2014). The samples were weighed using a semi-analytical scale with 0.01 g precision. For the first hour, the sample was weighed every 15 min. After the first hour, the sample was weighed every 30 min, until it reached a constant weight. Weight loss was standardized for moisture, using the following equation:

$$U_{adm} = \frac{(U_t - U_e)}{(U_o - U_e)},$$

where: U_{adm} is the dimensionless moisture; U_t is the slice moisture at time t; U_e is the equilibrium moisture; and U_0 is the initial moisture.

A moisture loss vs time equation was adjusted using the modified Page model (Equation 2). This model was selected due to its statistical performance to describe drying kinetics. It uses a nonlinear regression, where squared errors are minimized using the Gauss-Newton method.

$$U_{adm} = e^{-(kt)^n},$$

where: k is the constant drying rate value; t is the time; and n is a shape parameter.

The equation fitting to the experimental data was evaluated using the correlation coefficient (R^2), chi-square (χ^2), and root mean square error (RMSE). Treatments were compared using the k-values, which indicate the drying rate. The higher is the k-value, the faster will be the drying process.

The color parameters were evaluated in the central region of the samples. CIELAB color space parameters were determined using D-65 diffuse illumination of a colorimeter (CR-400, Konica Minolta). The parameters L* (brightness), a* (redness), and b* (yellow) were calibrated using a standard white plate. The color differential (ΔE) was calculated using the CD as reference. The following equation was applied:

$$\Delta E = \sqrt{(a^* - a_o^*)^2 + (b^* - b_o^*)^2 + (L^* - L_o^*)^2},$$

where: L*, a*, and b* are the CIELAB parameters of the pretreated and dried samples; and L_0*, a_0*, and b_0* are the parameters of the CD treatment.

Hue angle and chroma (C*) were calculated with following equations, respectively.

$$\text{hue} = \tan^{-1}\left(b^*/a^*\right), \text{ and } C^* = \sqrt{(a^2 + b^2)},$$

The extraction and analysis of betalains were carried out based on a modified method proposed by Prieto-Santiago et al. (2020). A portion of 0.5 g of each sample was put into polyethylene Falcon tubes, and 5 mL of a hydroalcoholic ethanol solution (1:1, v/v) were added. The system was vigorously mixed in a vortex equipment (model K45-2818, Kasvi) for 30 min, and the supernatant (extract) was collected. If red pigment was observed in the beetroot in the solution, the solid beetroot left was collected from the solution, and a new 5 mL of the hydroalcoholic ethanol solution was added and mixed for 30 min, and the supernatant was collected again. This procedure was repeated until no red pigment was observed in the solid residue. All extracts obtained from the same sample were mixed, and their absorbance measured at 476, 538, and 600 nm. The betaxanthin and betacyanin concentrations were calculated according to following equation .

$$\text{Betaxanthis or betacyanins} = \frac{A \times DF \times MW \times 1,000}{\varepsilon \times L},$$

where: A is the λmax absorption (476 and 536 nm for betaxanthin and betacyanin, respectively) corrected by the absorption at 600 nm; DF is the dilution factor; MW is the molar weight (339 and 550 g mol^{-1} for betaxanthin and betacyanin, respectively); ε is the molar absorptivity (48,000 and 60,000 L mol^{-1} cm^{-1} for betaxanthin and betacyanin, respectively); and L is equal to the path length (cm).

The final data was expressed in milligrams of betalains per gram of dry beetroot (mg betaxanthins or betacyanin/g d.b.).

The analyses were performed using a scanning electron microscope equipped with an energy dispersive spectrometry (EDS) detector (EVO 10,

Zeiss), employing the high vacuum method and 10 kV acceleration voltage. The magnification is described as Mag. The samples were subjected to chemical fixation with glutaraldehyde at 2.5% for 24 hours; then, they were washed in phosphate buffer solution and dehydrated using alcohol at 50, 60, 70, 80, 90 and 99% (Castro, 2001; Dal Magro et al., 2016).

Data was checked for normality of the residues, homoscedasticity, and independence of residues, and the Shapiro-Wilk's test (α=0.05), the Hartley's maximum F-test (α=0.05), and the Durbin-Watson's test (α=0.05) were used, respectively. The R software v.4.3.0 (R Core Team, 2023) was used for assumption checking. They were met. Two-way analysis of variance was used to compare the k-values and n-values means. The Fisher's exact test was used to compare the treatments (α=0.05) individually. The software Statistica 10.0 (StatSoft Inc., Tulsa) was used. The other results were subjected to two-way analysis of variance, and the means were compared by the Tukey's test, at 5% probability.

Results and discussion

The drying curves of beetroots subjected to each treatment show a classical drying behavior for all samples (Figure 1). Slices pretreated with cellulase (CP), dry control (CD) and wet control (CW) showed similar drying curves. Slices pretreated with pectinase (PP) presented slower moisture decay.

The plot of drying rate as a function of moisture for each treatment is presented (Figure 2). Moisture was calculated on dry basis as a dimensionless variable. Dry basis was determined using a sample dried at 105°C until it reached constant weight. High moisture is the starting point of the drying process. It decreases until reaching moisture 0, that is, when it is completely dry. In general, it was observed that drying rates increased at the beginning of the process until reaching a maximum drying rate of 0.5 g of water per minute, at moisture 3 or 4. All treatments showed similar behavior. According to Fellows (2018), at the beginning of the drying process, drying rates increase until reaching the wet bulb temperature, when the food surface is surrounded by steam, and the maximum drying rate is observed. At this period, the rate of water removed from the surface is equal to the water migration from the food's interior to the surface. When the water supply is no longer enough to maintain the food surface surrounded by steam, the food surface temperature raises and starts to dry. While drying, drying rates decay until reaching zero values.

The moisture reduction (Figure 1) and drying rates (Figure 2) show that PP was related to a high loss of water in the beginning of the drying process. Pectinase was effective to remove the water not linked to the

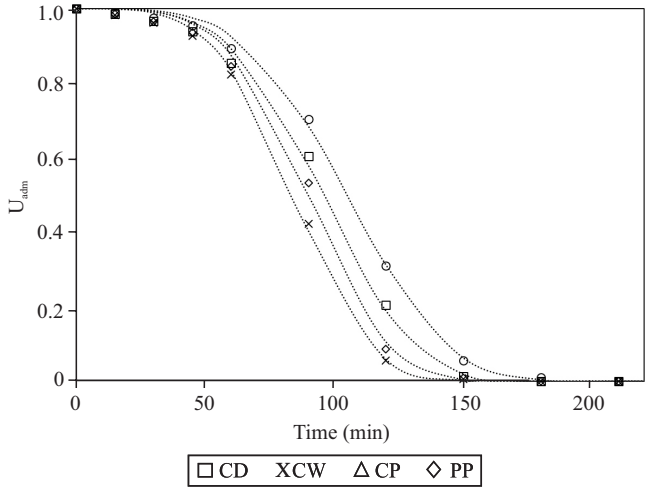

Figure 1. Drying curves of beetroot (*Beta vulgaris*) slices subjected to the following pretreatments: CD, dry control; CW, wet control; CP, cellulase pretreatment; and PP, pectinase pretreatment. Dotted lines are adjusted curves of a modified Page model.

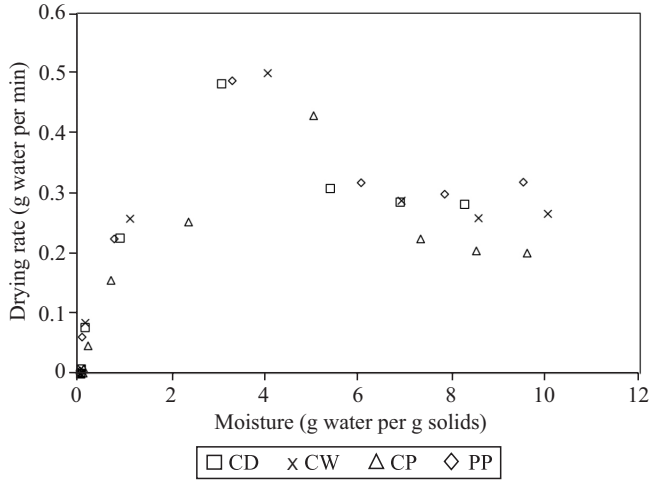

Figure 2. Drying rates vs moisture of beetroot (*Beta vulgaris*) slices subjected to the following pretreatments: CD, dry control; CW, wet control; CP, cellulase pretreatment; and PP, pectinase pretreatment.

plant tissue, although it did not remove water strongly bounded to the food matrix. The latter happens in the end of the drying process.

The CP showed similar behavior to that of the dry control (CD) and wet control (CW). This behavior indicates that the tissue was not affected enough to change the cell turgidity and the cell wall organization, in order to hinder the water diffusion. The hydrolysis softens the plant fibers and enhances their water retention capacity (Yoshida & Prudencio, 2020). This enhanced capacity does not let water to break bonds with the plant tissue, making it difficult to lose water along the drying process. Additionally, monosaccharides and soluble oligosaccharides, which are formed after hydrolysis, have more water-binding sites than polysaccharides (Yoshida & Prudencio, 2020). This fact adds another barrier to moisture loss, when using enzyme pretreatments. Finally, the solid gain (incorporation of solute) during the enzymatic process increases the interaction of water with the plant material, interfering in the outflow of water during the drying process (Macedo et al., 2021).

A modified Page model was used to evaluate the experimental data. The fit indicators of the model to express the U_{adm} reduction through time shows that the model presented a good fit and that R^2 was close to 1 (Table 1). The χ^2 values obtained were below the critical value of $\chi^2(3, 84) = 7.815$, $\alpha=0.05$, that is, no significant differences were observed between the data and the model. RMSE was below 0.004. Afrin et al. (2022) reported similar results of fit indicators.

The estimated k-values and n-values are presented (Table 2). A n-value > 1 indicates that the semilogarithmic curve has a downward concavity, a n-value < 1, an upward concavity, and a n-value = 1, an exponential distribution. The n-values for all treatments were higher than 3.871, which indicates a downward concavity curve (Table 2). The moisture declined slowly at the beginning of the process. The results showed that the enzymatic pretreatments did not affect the drying performance ($p>0.05$). In fact, pectinases delayed the drying process, in comparison with to the control treatments ($p=0.069$). These results agree with those observed in the drying curves (Figure 1).

The control treatments (CD and CW) show intact tissue structures (Figures 3 A and 3 B). However, in both enzymatic pretreatments (PP and CP), degradation was observed in the beetroot tissue structures (Figures 3 C and 3 D). A similar result was reported for apple treated with enzymes (Dongowski & Sembries, 2001). Results indicate that cellulose breakdown in beetroot does not facilitate water removal during the hot air-drying process. Yoshida & Prudencio (2020) reported that hydrolysis with carbohydrase mixture improved the water retention capacity in okara (fibrous residue from soymilk and tofu production), due to the presence of more porous and fragmented particles. The hydrolysis decreased the insoluble fiber content and increased the soluble fibers and sugar contents. This fact may explain the observed results in the drying curves (Figure 1) and in the k-values and n-values (Table 2).

Dried beetroots analysis for color parameters showed that L* values of CP were similar ($p>0.05$) to those of the control treatments (Table 3). The PP resulted in darker dried beetroot in comparison with those of the control treatments ($p<0.05$). This result is probably due to the free sugars released during the enzymatic treatment (Yoshida & Prudencio, 2020). These results reinforce the hypothesis of the increase of free sugars hindering the outflow of water in the drying process.

The a* values are related to the reddish tons, which it is typical in betalain-rich foods. The CP had the lowest

Table 1. Fit indicators R^2, chi-square (χ^2), and standard error of the mean (SEM) of data adjustment to a modified Page model used to describe the drying kinetics of beetroot (*Beta vulgaris*) slices.

Treatment	R^2	χ^2	RMSE
Dry control (CD)	0.993	0.00172	0.0025
Wet control (CW)	0.997	0.00077	0.0036
Pectinase pretreatment (PP)	0.996	0.00079	0.0032
Cellulase pretreatment (CP)	0.998	0.00033	0.0041

Table 2. Pretreatments k-values and n-values for beetroot (*Beta vulgaris*) slices based on the modified Page model fitting[(1)].

Treatment	k-values/min	n-values
Dry control (CD)	0.00950±0.0012a	3.871±0.406a
Wet control (CW)	0.01085±0.0011a	3.969±0.241a
Pectinase pretreatment (PP)	0.00866±0.0006a	3.893±0.232a
Cellulase pretreatment (CP)	0.01017±0.0007a	4.068±0.232a

[(1)]Means followed by equal letters in the same column, do not differ between treatments, at 5% probability.

a* values ($p<0.05$). The PP did not differ ($p>0.05$) from the wet control (CW) for a* values. The hue angle in the PP was higher than those of the other treatments ($p<0.05$), while the CP and control treatments (CD and CW) did not differ from each other ($p>0.05$) for this parameter. The PP showed higher tones of red (a*) than those of the other treatments. For the C* values, the PP and the wet control (CW) showed higher values than that of the CP and of the dry control (CD). The difference in colors (ΔE) used dry control (CD) as color reference. The CP did not differ from the wet control ($p>0.05$). However, the PP had the highest color difference ($p<0.05$). The ΔE results agree with the hue results. The results of the color parameters indicate that the pectinase pretreatment (PP) had more significant changes, which may compromise its use in future studies.

For the contents of betacyanins and betaxantins No significant differences were observed between treatments ($p>0.05$) (Table 4). These results indicate

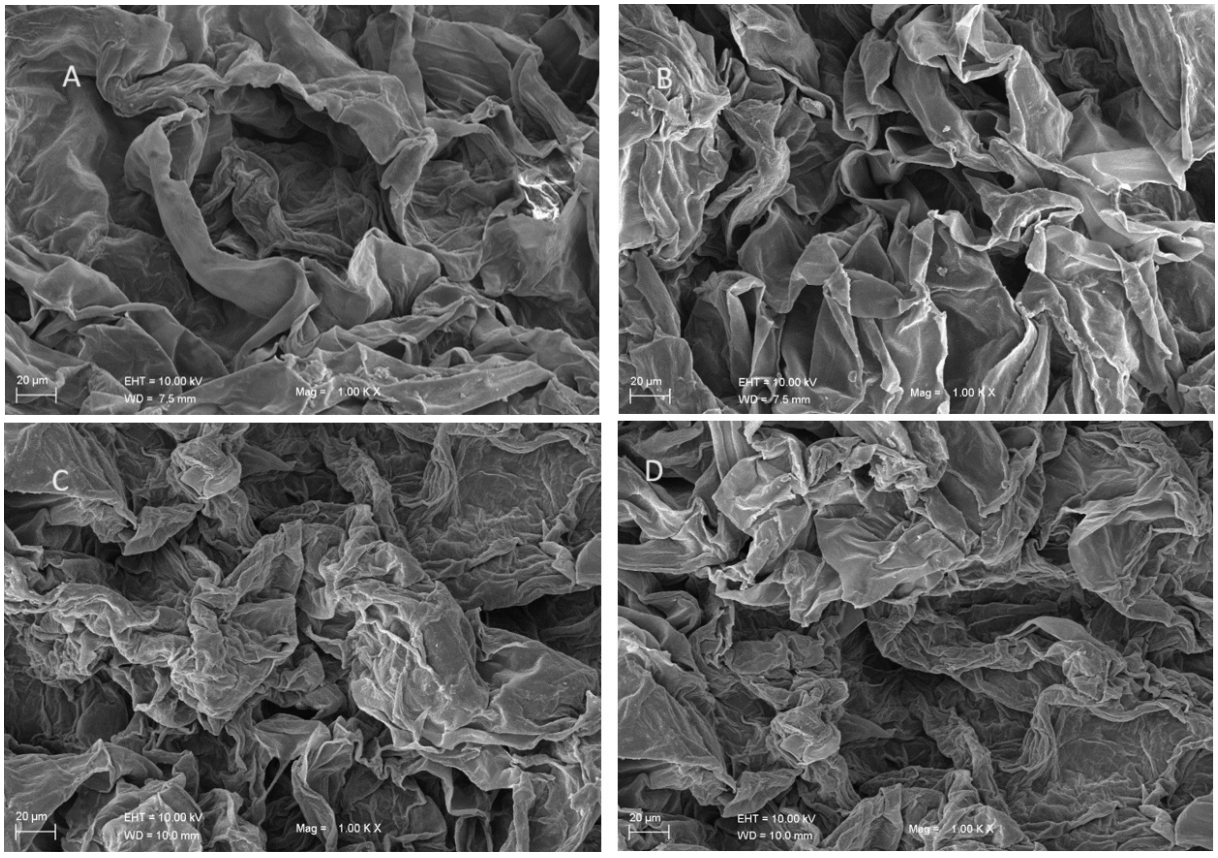

Figure 3. Scanning electron microscopy images of beetroot (*Beta vulgaris*) subjected to the following pretreatments: A, dry control (CD); B, wet control (CW); C, pectinase pretreatment (PP); and D, cellulase pretreatment (CP). Magnification: 1,000X.

Table 3. CIELAB color space parameters of beetroot (*Beta vulgaris*) slices subjected to the treatments dry control (CD), wet control (CW), pectinase pretreatment (PP), and cellulase pretreatment (CP), before the drying process at 60ºC[(1)].

Treatment	L* values	a* values	b* values	Hue	C	ΔE
CD	30.23±0.88ab	19.34±0.39b	11.52±0.95ab	1.47±0.01b	22.51±1.08bc	-
CW	32.58±2.28a	21.02±1.52ab	12.79±0.88a	1.43±0.05b	24.61±0.52a	1.67±0.02b
PP	22.95±1.11c	21.93±0.48a	10.32±0.48b	1.96±0.04a	24.23±1.21ab	8.09±0.89a
CP	26.56±2.57bc	17.53±0.52c	10.41±0.52b	1.48±0.05b	20.38±0.55c	2.91±1.14b

[(1)]Means followed by equal letters in the same column, do not differ between treatments, at 5% probability.

Table 4. Contents of betacyanins, betaxantins and total betalains of beetroot (*Beta vulgaris*) slices subjected to four treatments, before the drying process at 60ºC[(1)].

Treatment	Betacyanins (mg g^{-1})	Betaxantins (mg g^{-1})	Total betalains (mg g^{-1})
Dry control (CD)	78.74±57.18a	78.74±57.18a	114.04
Wet control (CW)	57.55±1.74a	56.48±3.72a	127.33
Pectinase pretreatment (PP)	43.34±5.86a	44.34±6.89a	87.68
Cellulase pretreatment (CP)	51.56±3.49a	49.38±10.89a	100.94

[(1)]Means followed by equal letters in the same column, do not differ between treatments, at 5% probability.

that pigment contents did not change. The enzymatic pretreatments did not affect the pigment contents.

Enzymatic pretreatments did not enhance the drying kinetics. However, they did not reduce the content of functional compounds, which is a positive result. The present study suggests that future research should focus on optimizing cellulase pretreatment.

Conclusions

1. Pectinase and cellulase pretreatments did not increase the drying performance of beetroot (*Beta vulgaris*) slices.
2. Cellulase pretreatment does not change the beetroot color; however, pectinase pretreatment does it.
3. Pectinase and cellulase pretreatments change the plant tissue structure.
4. Cellulase pretreatment is promising, since it does not change the beetroot color or betalain concentrations.

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