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A natural clouding agent from orange peels obtained using polygalacturonase and cellulase

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Abstract

Clouding agents (CAs) provide high cloud stability when they are diluted and added to fruit beverages. Natural CA could also give the visual appearance and sensorial aspect of a natural cloudy fruit juice beverage. In addition, natural CAs are preferred over the synthetic ones by consumers. Therefore, the main objective of this study was to obtain a natural CA from orange peel that provided high cloud stability of fruit beverages. The treatment included the use of polygalacturonase (PG) and cellulase (C) to hydrolyze most of the peel components. Orange peels treated with 69 mg/kg of C and 90 μl/kg of PG during 80 min at 48 °C led to a CA that provided a cloudy stable solution on a fruit beverage. This CA presented a cloud stability of 94.7 days that represented a turbidity reduction of 9.1% after 9 days under fridge temperatures. Otherwise, the treatment conditions to get the highest cloud stability were not coincident to those necessary to preserve the color or to obtain the greatest extraction yield of the CA.

Keywords: Clouding agent; Orange peel; Cloud stability; Enzymatic treatment; Surface response

1. Introduction

Citrus cloud is a complex mixture of cellular organelles and membranes, chromatophores, oil droplets, flavonoid crystals, and cell wall fragments including pectin, cellulose, and hemicellulose. The cloud provides turbidity, flavor, aroma and the characteristic color of citrus juices to fruit derived beverages (Baker & Cameron, 1999). But, natural citrus cloud is known to flocculate and sediment with time, and it cannot be redispersed to match the original cloud even by shaking, because irreversible aggregates are formed. Therefore, the majority of non-alcoholic beverages contain a clouding agent (CA) that provides turbidity. This CA also gives the characteristic flavor, color and mouthfeel to the beverage (Jasentuliyana, Toma, Klavons, & Medora, 1998).

Some years ago the use of synthetic CAs such as brominated vegetable oil (BVO) was widely extended but, nowadays, their use is restricted. Moreover, consumers are asking for products with natural ingredients. Thus, CAs from natural sources are being studied as additives for fruit beverages.

Klavons, Bennett, and Vannier (1992) developed a process to obtain a stable cloud by adding isolated soy protein to a solution of pectin. Afterwards, Jasentuliyana et al. (1998) optimized the ratio of pectin to isolated soy protein fraction that gave the most stable cloud. Soy protein also was used as a CA by Garti, Aserin, and Azaria (1991) in a formulation of an orange oil drink based on naturally occurring ingredients. Moreover, the use of hydrolyzed starch from different sources of CA was studied (Abbas, Bishop, Mackey, Patil, & Wilson, 1990).

On the other hand, when the citrus fruits are squeezed, about 50% of their weight is discarded as

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waste peel, membrane, juice vesicles and seeds (Crandall, Matthews, & Baker, 1983). Then obtaining by-products from these wastes may increase the economic yield of the citrus juice industries. Citrus by-products are commonly used to fortify animal fodders (Braddock, 1999), but also the citrus peels are used to obtain citroflavonoids (Arriaga, 1989), aromatic components, carotenoids (Lafuente, 1980) and dietary fiber concentrates (Grigelmo-Miguel & Martin-Belloso, 1999). Thus, these wastes may be used as a source of CA for citrus beverages. The CA may be obtained from citrus wastes, after extracting the proteins by addition of salt (Cameron, Baker, & Grohmann, 1997). The CA also can be obtained by the addition of alcohol or by fermentation with bakers and brewers yeast (Sreenath, Crandall, & Baker, 1995). These extraction processes reduced the soluble solids by almost 50% and 72% for the fermentation and alcohol extraction, respectively. Moreover, the alcohol extraction also removed all the natural color of the orange peels.

A CA from orange peels without reduction of soluble solids and color from the original peels can be obtained by an enzymatic treatment. The treatment also produced an 83% reduction in viscosity when compared with untreated sample (Sreenath et al., 1995). Lashkajani (1999) also used commercial pectolytic and/or cellulytic enzymes to extract CAs from citrus peels. The CAs from orange peels obtained by commercial enzymes provided high cloud stability, but the individual effect of each enzyme is not completely known. Therefore, the study of individual enzymes may contribute to the knowledge on the influence of each enzyme on the CA extraction, and would help to improve CA properties.

Thus, the purpose of this work was to determine the treatment conditions to reach the maximum cloud stability of a CA from orange peels using pure polygalacturonase and cellulase. Color parameters of the CA and its process extraction yield were also studied.

2. Materials and methods

2.1. Materials

Orange peels were supplied by a local fruit juice industry, Indulleida S.A. (Alguaire, Lleida, Spain). Orange peels were divided in lots of 0.5 kg and every lot was packed under a 50% of vacuum with an EGAR VAC® Basic-9 Digital Compensated vacuum machine (Egarvac S.C.P. Terrasa, Barcelona, Spain) and treated at 90 °C for 1 min to inactivate all the natural enzymes. Then, the non-enzymatically active orange peels were characterized according to A.O.A.C. (1990) methods (Table 1) and frozen below –18 °C until the treatment for obtaining the CA was carried out.

Table 1
Characterization of orange peel

Parameter	Value
Soluble solids (°Brix)	7.1 ± 1.2
pH	3.93 ± 0.03
Total acidity (g of citric acid/100 ml)	0.29 ± 0.03
Formol index	34.0 ± 2.4
Humidity (%)	85.9 ± 1.6
Fat (%) in DM	1.55 ± 0.17
Protein (%) in DM	6.16 ± 0.23
Ashes (%) in DM	3.29 ± 0.19
Carbohydrates (%) in DM	89.0 ± 1.1
Soluble fiber (%) in DM	
Neutral sugars	3.8 ± 0.3
Uronic acids	1.04 ± 0.18
Insoluble fiber (%) in DM	
Neutral sugars	17.1 ± 1.6
Uronic acids	7.1 ± 0.9
Klason lignin	3.2 ± 0.4
Pectin (%) in DM	17 ± 4
Essentials oils (ml/kg)	1.45 ± 0.16
Color	
a^*	8.52 ± 0.22
b^*	52.3 ± 0.8
L^*	70.2 ± 0.7

Values are means ± SD.
DM, dry matter.

2.2. Obtaining clouding agent

A surface experiment design with four factors was used to establish the treatment conditions. The factors were the treatment temperature (T), the treatment time (t) and the concentration of each enzyme [polygalacturonase (PG) (EC 3.2.1.15) (P-0764 Sigma Chemical Co., St. Louis, MO, USA) and cellulase (C) (EC 3.2.1.4) (C-1184 Sigma Chemical Co., St. Louis, MO, USA)] (Table 2).

A lot (0.5 kg) of the non-enzymatically active orange peels were taken from the freezer. These citrus peels were added with 300 ml of warm water, and the mixture was ground with a blender in slices of 3–5 mm approximately. Peels were heated to the suitable temperature in a thermostatic bath (Clifton, Nickel-Electro Ltd., North Somerset, England) and then the enzymes were added. The mixture was stirred during the heating process and the enzymatic treatment. When the treatment finished, the temperature of samples was suddenly increased up to 90 °C and maintained for 2 min to reduce completely the enzymatic activity.

The mixture was filtered at room temperature with a strainer to separate the huge particles because they could interfere on the sedimentation. Then, the samples were centrifuged (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, CA, USA) at 3840g for 15 min and filtered under vacuum conditions with an Albet® qualitative analysis filter paper (reference 400). Afterwards, the samples were concentrated to more than 40° Brix by a rotatory evaporator R-3000 (B.U.C.H.I Labortechnik AG., Flowil, Switzerland) at 50 °C to obtain a concentrated CA.

Table 2
Experimental design of response surface, showing the level of the factors used in each experiment: treatment temperature, polygalacturonase (PG), and cellulase (C) and treatment time

Experiment number	Temperature (°C)	PG (μl/kg)	C (mg/kg)	Time (min)
1	50	90	30	40
2	30	90	30	40
3	30	210	30	40
4	50	210	30	40
5	30	90	90	40
6	50	90	90	40
7	50	210	90	40
8	30	210	90	40
9	30	90	30	80
10	50	90	30	80
11	50	210	30	80
12	30	210	30	80
13	50	90	90	80
14	30	90	90	80
15	30	210	90	80
16	50	210	90	80
17	40	50	60	60
18	40	250	60	60
19	40	150	10	60
20	40	150	110	60
21	40	150	60	27
22	40	150	60	93
23	23	150	60	60
24	57	150	60	60
25	40	150	60	60
26	40	150	60	60
27	40	150	60	60

The most adequate treatment conditions to obtain the CA from orange peels were established through the highest cloud stability provided by the CA. Color parameters as well as the CA extraction yield were also studied.

2.3. Cloud stability measurement

A 0.5° Brix solution of the orange peel CA was prepared to determinate the cloud stability. The turbidity (HACH RATIO TURBIDIMETER 18900, Loveland, Colorado, USA) of the solution was determined during 10 days of storage at 5 °C. The solution was added with 0.05% of sodium azide to prevent the grown of microorganisms that could increase the turbidity (Pozsar-Hajnal & Polacsek-Racz, 1975).

The evolution of the turbidity with the storage time was fitted to an exponential model (Eq. (1)), and the S (days) of the CA was defined as the inverse of the kinetic constant (k, days^{-1}) (Eq. (2)).

$$T = T_0 \times e^{-kt} \tag{1}$$

$$S = \frac{1}{k}, \tag{2}$$

where T is the relative turbidity (%), T_0 is the intercept of the curve (%), and t is the storage time (days).

2.4. Color measurement

The CIELab coordinates (L^*, a^*, b^*) of the CA concentrate from orange peel were directly read with a spectrophotocolorimeter Color-Eye 3000 (Macbeth-Kollmorgen Ins. Corp., Newburgh, NY) with a D_{75} light source and the observer at 10°. The influence of enzyme concentrations, treatment temperature and time was performed on a 20° Brix dilution.

2.5. Extraction yield measurement

The yield of the CA extraction (Y) was expressed as the quantity of CA at 40° Brix obtained from fresh orange peel expressed in (%), according to the Eq. (3).

$$Y = \frac{m}{M} \cdot 100, \tag{3}$$

where m is the amount of CA obtained at 40° Brix (g) and M is the weight of peels (g). The extraction yield was expressed as the amount of the CA obtained at 40° Brix because more concentration was difficult to achieve with some treatment conditions.

2.6. Statistics

Each processing condition was assayed in duplicate and the determinations also were performed in duplicate. Therefore, the results were averages of four measurements.

Cloud stability, L^*, a^*, b^* and the extraction yield were subjected to a regression analysis using the following response surface model:

$$\begin{aligned} X = {} & b_0 + b_1 T + b_2 t + b_3 \mathrm{PG} + b_4 \mathrm{C} + b_5 T \cdot t + b_6 T \\ & \cdot \mathrm{PG} + b_7 T \cdot \mathrm{C} + b_8 t \cdot \mathrm{PG} + b_9 t \cdot \mathrm{C} + b_{10} \mathrm{PG} \\ & \cdot \mathrm{C} + b_{11} T^2 + b_{12} t^2 + b_{13} \mathrm{PG}^2 + b_{14} \mathrm{C}^2, \end{aligned} \tag{4}$$

Table 3
Code table for the values of treatment temperature, polygalacturonase (PG), cellulase (C) and treatment time

	Real value	Code	Real value	Code	Real value	Code
Temperature (°C)	50	1	40	0	30	−1
PG(μl/kg)	210	1	150	0	90	−1
C (mg/kg)	90	1	60	0	30	−1
Time (min)	80	1	60	0	40	−1

where X is the parameter of study, T is the treatment temperature, t is the treatment time and b_0 to b_{13} are regression coefficients.

The significant coefficients of the regressions were identified by an Analysis of Variance with a $p \leqslant 0.05$. These analyses were performed by Statgraphics plus version 3.0 for Windows package (Statistical Graphics Co., Rockville, MD, USA, 1997). To perform the statistical analysis, the levels of the factors were coded (Table 3) to normalize and standardize the variance of the data.

3. Results and discussion

3.1. Cloud stability provided by the CA

The enzymatic treatment of the orange peels with PG and C produced a CA with a S up to 94.7 days using 45 °C, 80 min, 69 mg of C/kg and 90 μg of PG/kg as treatment conditions, which represented a turbidity maintenance of 90.9% after 9 days of fridge storage. Similar results were observed after a fermentation treatment of citrus peels with baker's yeasts (Sreenath et al., 1995) (95%). The turbidity maintenance of the CA obtained in the present work was higher than that obtained after a pectolytic treatment of citrus peels (65%) using a commercial enzyme (Ultraenzym) at 25–30 °C for 1 h (Sreenath et al., 1995). Turbidity maintenance also was higher than that found by the latter authors on a CA extracted with ethanol 95% from orange peel (79%). Moreover, the turbidity maintenance of a CA from orange peels was 80% after an 8-week storage test when the clouding agent was added at 1.0% w/w to a standard drink base (Crandall et al., 1983). Furthermore, Jasentuliyana et al. (1998) defined the cloud stability as the percentage that represented the height of the cloud phase on the total height of the suspension, and obtained a CA of soy protein with a retention of 97% of the cloud stability after 28 days of storage at 4 °C.

The S of the CA was highly affected by the treatment temperature, C concentration and treatment time. However, PG concentration did not affect significantly ($p > 0.05$) the S. Eq. (5) expresses the influence of significant parameters to S.

$$S = 83.99 + 8.17 \cdot T + 8.25 \cdot t + 11.24 \cdot C - 9.17 \cdot T^2 - 19.07 \cdot C^2 \quad R^2 = 63.9 \tag{5}$$

A maximum of S was observed when the treatment was performed at 45 °C (Fig. 1), that is within the range where PG and C are more active (45–50 °C) (Pozsar-Hajnal & Polacsek-Racz, 1975; Solehah et al., 1994). Moreover, the higher the treatment time the higher S was observed in the range of 40–80 min (Fig. 1). Then, a treatment of 80 min was the most adequate to obtain the CA with the greatest S. Furthermore, a concentration of C of 69 mg/kg lead to the CA with the greatest S (Fig. 1). Otherwise, as PG concentration did not influence the cloud stability, the lowest concentration (90 μl/kg) was enough to achieve the highest S.

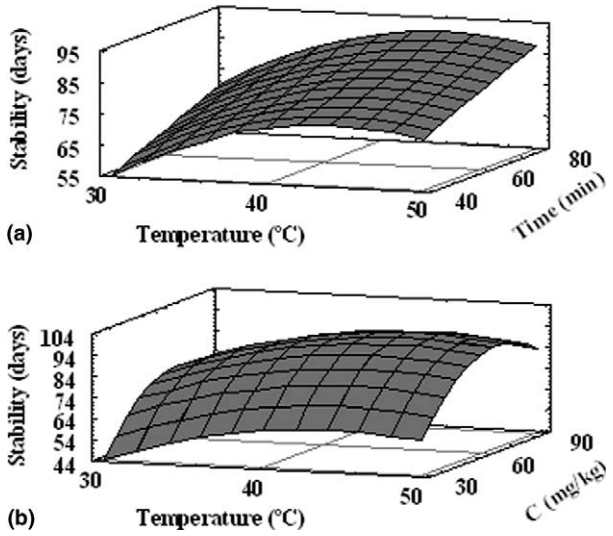

Fig. 1. Clouding agent cloud stability as a function of: (a) treatment temperature and time with the addition of 69 mg/kg of C, (b) treatment temperature and C concentration during 80 min. C, Cellulase concentration.

3.2. Clouding agent color

The L^* and b^* color parameters were only affected significantly ($p < 0.05$) by the treatment temperature. However, the a^* parameter changed significantly with the temperature, PG and C concentrations and their relationship was expressed by Eq. (6).

$$a^* = 2.128 - 0.373 \cdot T - 0.290 \cdot \mathrm{PG} - 0.282 \cdot \mathrm{C} - 0.213 \cdot T \cdot \mathrm{PG} + 0.172 \cdot T \cdot \mathrm{C} + 0.312 \cdot \mathrm{PG}^2 - 0.226 \cdot \mathrm{PG} \cdot \mathrm{C} \quad R^2 = 65.7 \tag{6}$$

In all the samples, the CA showed a positive a^* and b^* value, that is a red and yellow coloration, respectively. Orange juice presents a b^* value of approximately 23 and a^* near to 2.5 (Ayhan, Yeom, Zhang, & Min, 2001). Therefore, as the CA bring color to the beverages and to avoid the addition of any colorant, the a^* and b^* values of the CA should be the maximum possible to reach a similar coloration to an orange juice after its dilution on citrus based beverage. In the same way, L^* also has to be as high as possible to give the appropriated fresh appearance to the final beverage formulation.

The values of L^* ranged from 35 to 49, and the b^* values were between 20 and 36 within treatment temperatures of 30 and 50 °C. Moreover, the CA presents the maximum a^* (3.6) when orange peels were treated at the lowest treatment temperature (30 °C). The reduction of a^* due to an increase of the temperature was lower

when the C concentration was augmented, having no influence on the a^* value when the C concentration used to carry out the extraction was 90 mg/kg. The highest value of L^* and b^* were also reached at 30 °C that is far away of the temperature range where PG and C are more active (45–50 °C) (Pozsar-Hajnal & Polacsek-Racz, 1975; Solehah et al., 1994). Therefore, high temperature degrades the color of the CA and the enzyme actuation did not produce the extraction of color components from orange peels which provides color to the CA.

As occurred with L^* and b^* parameters, the treatment time did not affect the value of a^*. Finally, the CA with the highest a* was obtained with the lowest enzyme concentrations, 90 μl/kg of PG and 30 mg/kg of C, and a rise of the enzyme concentration produced a depletion on the a^* value. The effect of the C concentration on the a^* was more noticeable at temperature and PG concentration of 30 °C and 90 μg/kg, respectively (Fig. 2).

The treatment performed at 30 °C with 30 mg/kg of C and 90 μl/kg of PG, that are the most appropriate conditions to preserve the color, produced a reduction of the S of the CA to 50.6 days. This S represented only

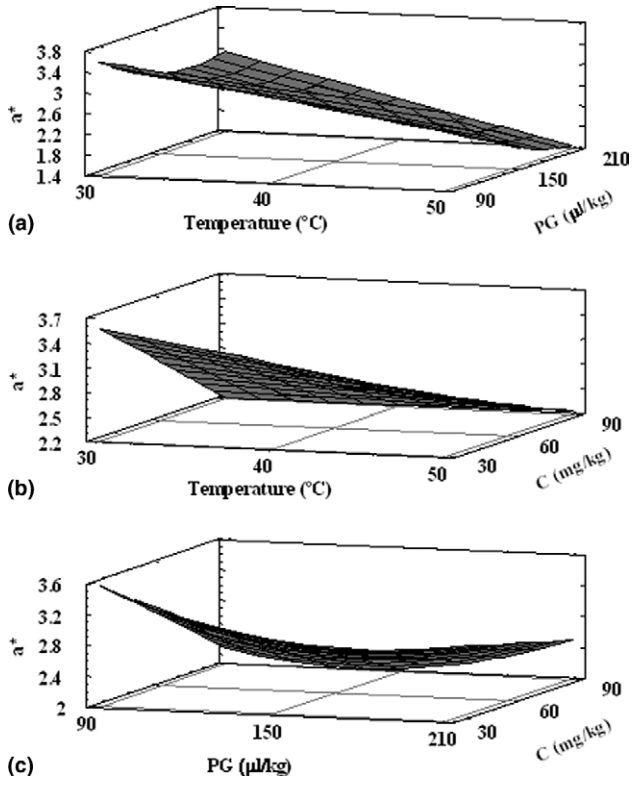

Fig. 2. Evolution of the a^* parameter of the clouding agent from orange peels with: (a) PG concentration and treatment temperature with the addition of 30 mg/kg of C, (b) C concentration and treatment temperature with the addition of 210 μl/kg of PG, (c) PG and C concentration at 30 °C. PG, Polygalacturonase concentration, C, Cellulase concentration.

53% of the greatest S of CA obtained. On the other hand, the value of the a^* b^* and L^* parameters suffered a reduction to 2.7, 25 and 39, respectively, when the enzymatic treatment was carried out at the conditions that leads to a CA with the maximum S.

Although, no more studies were found about CAs color, the effects of the treatment conditions on color parameters obtained from the present study can be compared to results from works focused on temperature and time effects on some fruit products. A depletion on L^* of the CA occurred when the temperature was increased. Similar behavior of L^* was observed for peach puree (Ávila & Silva, 1999). Otherwise, a small rise of L^* was appreciated after a heat treatment in grapefruit and apple juice (Genovese, Elustondo, & Lozano, 1997; Lee & Coates, 1999).

The treatment time and temperature did not affect significantly the a^* parameter after a heat treatment of 91 °C in red grapefruit (Lee & Coates, 1999). Moreover, an increase of the a^* value was found after a thermal treatment of more than 100 °C on peach puree (Ávila & Silva, 1999). These temperatures are higher than the used in the process to obtain the CA, where temperatures near 50 °C produced an increase of a^*.

As observed in the CA from orange peels, the CIE b^* decreased for peach puree when temperature of a heat treatment increased (Ávila & Silva, 1999). However, in red grape, the CIE b^* value increased after a heat treatment of 10 s at 91 °C (Lee & Coates, 1999).

Therefore, the influence of the treatment temperature on color parameters change with the studied product.

3.3. Effects of treatment conditions on the extraction yield

The extraction yield of the CA from orange peel significantly depended ($p < 0.001$) on the treatment temperature, as well as the PG and C concentrations. However, the treatment time presented no significant effect ($p > 0.05$) on the yield extraction of the CA. The combined effect of treatment temperature and enzymes concentrations on extraction yield (Y, %) was described by the Eq. (7).

$$Y = 13.0936 - 0.5980 \cdot T + 0.7860 \cdot \mathrm{PG} + 1.2046 \cdot \mathrm{C} + 0.8332 \cdot T^2 - 0.4873 \cdot T \cdot \mathrm{PG} - 0.8422 \cdot T \cdot \mathrm{C} - 0.3560 \cdot \mathrm{PG}^2 + 0.8862 \cdot \mathrm{PG} \cdot \mathrm{C} + 1.3973 \cdot \mathrm{C}^2 \quad R^2 = 85.5. \tag{7}$$

The treatment conditions to reach the highest extraction yield (18.2%) were the use of 210 μl/kg of PG and 90 mg/kg of C at 30 °C. The treatment time did not produce any effect within 40 and 80 min. The yield was reduced with a rise of temperature, then, the lowest temperature (30 °C) led to the greatest extraction yield. However, the effect of the treatment temperature was

different depending on the level of PG and C concentration. The reduction of the yield with the increase of temperature was higher when the concentrations were 210 μl/kg of PG and 90 mg/kg of C, than using 90 μl/kg of PG and 30 mg/kg of C. Moreover, an increase of C concentration affected more the extraction yield than an increase of PG concentration. However, the effect of the PG concentration was higher when the C applied was the greatest (Fig. 3).

PG is a depolymerizing enzyme that degrades by hydrolysis the glucoside bonds of the main chain of polygalacturonic acid, and C enzyme hydrolyzes the β-1,4 linkages in cellulose (Schülein, 2000; Solehah, Balaumani, Das, & Amiza, 1994). In consequence, both enzymes reduce the weight of the food molecules. Therefore, the highest enzyme concentrations were required to reach the maximum extraction yield.

The treatment conditions that permitted to reach the maximum extraction yield produced a CA with 67 days of *S*. However, the main function of the CA is to provide cloud appearance to the beverages. Thus, *S* is the most important parameter to decide the appropriate conditions to perform the extraction of a CA from orange peel. A 12.2% of extraction yield was achieved when the treatment conditions to obtain the CA with the highest *S* were used. Moreover, the increase of the concentration of PG up to 210 μl/kg increased the extraction yield to 13.8%, maintaining the *S* in 94.7 days, the greatest value.

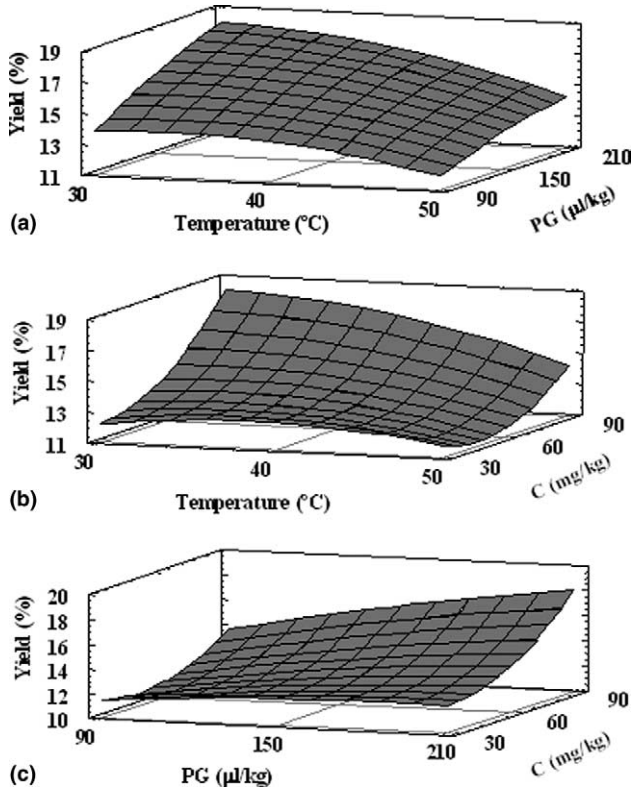

Fig. 3. Extraction yield of the CA from orange peels obtaining process as a function of: (a) PG concentration and treatment temperature with the addition of 30 mg/kg of C, (b) C concentration and treatment temperature with the addition of 90 μl/kg of PG, (c) PG and C concentration at 30 °C. PG, Polygalacturonase concentration, C, Cellulase concentration.

Crandall et al. (1983) reported a yield of 29–27% after a treatment with pectinase enzymes to extract a CA from orange peels whose composition is not described. The peels used to carry out the present study were picked up directly from the production line of a fruit juice factory, where the juice extraction process took out a large amount of pectins. The characteristics of the peels are shown in Table 1. In consequence, the differences between the obtained yield in the present work and the observed by Crandall et al. (1983) may be due to changes in the initial composition of peels and for the addition of different enzymes during the enzymatic treatment.

4. Conclusions

The use of PG and C to produce a CA from orange peels with high cloud stability is feasible. The CA provided the greatest cloud stability when the process was carried out at 45 °C during 80 min with the addition of 90 μl/kg of PG and 69 mg/kg of C. However, these conditions did not produce the highest extraction yield and neither the CA with the optimum color parameters. Therefore, treatment temperature, time and C concentration necessary to achieve a CA with the best visual appearance have to be lower than those used to get the CA with the highest *S*. Moreover, the treatment to obtain the CA that presents the appropriate *S* was performed with lower enzyme concentrations and higher treatment temperature than that to achieve the maximum CA extraction yield.

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References

A.O.A.C. (1990). *Official methods of analysis*. (16th Ed.). Washingnton, DC: Association of Official Analytical Chemists.

Abbas, I. R., Bishop, R. M., Mackey, W. J., Patil, S. K., & Wilson, J. E. (1990). Beverage clouding agent. US Patent 4 971,828.

Arriaga, F. J. (1989). Aprovechamiento de subproductos cítricos: Flavonoides. *Alimentación, Equipos y Tecnología*(Noviembre–Diciembre), 173–179.

Ávila, I. M. L. B., & Silva, C. L. M. (1999). Modelling kinetics of thermal degradation of colour in peach puree. *Journal of Food Engineering, 39*, 161–166.

Ayhan, Z., Yeom, H. W., Zhang, Q. H., & Min, D. B. (2001). Flavor, color and vitamin C retention of pulsed electric field processed orange juice in different packaging materials. *Journal of Agricultural and Food Chemistry, 49*, 669–674.

Baker, R. A., & Cameron, R. G. (1999). Clouds of citrus juices and juice drinks. *Food Technology, 53*(1), 64–69.

Braddock, R. J. (1999). *Handbook of citrus by-products and processing technology*. New York: Wiley, 35–207.

Cameron, R. G., Baker, R. A., & Grohmann, K. (1997). Citrus tissue extracts affect juice cloud stability. *Journal of Food Science, 62*(2), 242–245.

Crandall, P. G., Matthews, R. F., & Baker, R. A. (1983). Citrus beverage clouding agents. Review and status. *Food Technology*(december), 106–109.

Garti, N., Aserin, A., & Azaria, D. (1991). A clouding agent based on modified soy protein. *International Journal of Food Science and Technology, 26*, 259–270.

Genovese, D. B., Elustondo, M. P., & Lozano, J. E. (1997). Color and cloud stabilization in cloudy apple juice by steam heating during crushing. *Journal of Food Science, 62*(6), 1171–1175.

Grigelmo-Miguel, N., & Martin-Belloso, O. (1999). Characterization of dietary fiber from orange juice extraction. *Food Research International, 31*(5), 355–361.

Jasentuliyana, N., Toma, R. B., Klavons, J. A., & Medora, N. (1998). Beverage cloud stability wit isolated soy protein. *Journal of the Science of Food and Agriculture, 78*, 389–394.

Klavons, J. A., Bennett, R. D., & Vannier, S. H. (1992). Stable clouding agent from isolated soy protein. *Journal of Food Science, 57*(4), 945–947.

Lafuente, B. F. (1980). Frutos, productos hortícolas y derivados. *Revista de Agroquímica y Tecnología de Alimentos, 20*(1), 13–21.

Lashkajani, H. B. (1999). Method for producing a clouding agent. US Patent 5 965,177.

Lee, H. S., & Coates, G. A. (1999). Thermal pasteurization effects on color of red grapefruit juices. *Journal of Food Science, 64*(4), 663–666.

Pozsár-Hajnal, K., & Polacsek-Racz (1975). Determinations of pectinmethylesterase, polygalacturonase and pectic substances in some fruits and vegetables. *Acta Alimentaria, 4*(3), 271–289.

Schülein, M. (2000). Protein engineering of cellulases. *Acta Biochimica et Biophysica, 1543*, 239–252.

Solehah, A., Balaumani, V. T., Das, K., & Amiza, M. A. (1994). Enzymes for improved extraction and stabilization of colour and flavour of orange juice. *Journal of Food Science and Technology, India, 31*(6), 508–510.

Sreenath, K. H., Crandall, P. G., & Baker, R. A. (1995). Utilization of citrus by-products and wastes as beverage clouding agents. *Journal of Fermentation and Bioengineering, 80*(2), 190–194.