RECyT
Año 19 / Nº 28 / 2017 / 11–15

Extracción asistida por enzima de licopeno en pulpa de Guayaba (*Psidium guajava* L.)

Enzyme assisted extraction of lycopene in guava pulp (*Psidium guajava* L.)

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Resumen

El licopeno es un carotenoide con beneficios en la salud humana y de amplio uso en la industria farmacéutica y alimentaria. El objetivo de esta investigación fue evaluar la extracción de licopeno en pulpa de guayaba asistida por la enzima pectinasa. Se evaluaron parámetros como la concentración de enzima y el tiempo de incubación. Los resultados indicaron que la extracción enzimática en la pulpa de guayaba permitió aumentar el rendimiento de extracción del licopeno en un 38,65%, usando una concentración de pectinasa de 0.02% (v/w de substrato), después de 45 minutos de incubación, a 28 °C y pH de 4,5. Bajo estas condiciones, la concentración de licopeno extraído fue de 40,08 ug/g materia fresca. El tratamiento con pectinasa aumenta la biodisponibilidad del licopeno en pulpa de guayaba, ofreciendo una alternativa interesante en procesos de extracción de tan importante antioxidante.

Palabras clave: Fruta exótica; Licopeno; CIEL*a*b*; Pectinasa; Extracción.

Abstract

Lycopene is a carotenoid with known benefits on human health and of wide use in pharmaceutical and food industry. The objective of this research was to evaluate the extraction of lycopene in guava pulp assisted by the enzyme pectinase. Parameters such as enzyme concentration and incubation time were evaluated. The results indicated that the enzyme improved the extraction of lycopene from guava pulp. An increase in lycopene yield of 38.65% was obtained, with a pectinase concentration of 0.02% (v/w of substrate) after 45 min of incubation, at 28°C and pH of 4.5. Under these conditions, the concentration of lycopen extracted was 40,08 ug/g fresh material. Pectinase treatment increases the bioavailability of lycopene in guava pulp, offering an interesting alternative in extraction processes of such important antioxidant.

Key words: Exotic fruit; Lycopene; CIEL*a*b*; Pectinase; Extraction.

Introduction

Guava (*Psidium guajava* L) is a fruit known by its high content of vitamins (A, C, B1 y B3, and others), minerals such as calcium, phosphorus and iron; and antioxidants (carotenoids, phenolics and ascorbic acid) (1). Its lycopene content quantified in 4-6 mg/100g of edible portion doubles tomato content (2). Lycopene ($C_{40}H_{56}$) is an acyclic carotenoid with thirteen linearly arranged double bonds, eleven conjugated and two not conjugated, considered an efficient antioxidant although having no activity as provitamin A (3-5). As an antioxidant lycopene can prevent or delay oxidative damage of lipids, proteins and nucleic acids, generated by reactive oxygen species which include free reactive radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non-radicals such as hydrogen peroxide, etc (6). This effect is achived through the inhibition of the initiation and breaking the chain propagation, or suppressing the formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, andquenching superoxide and singlet oxygen (6). Thus, this antioxidant not only presents benefits for human health, but also to the food industry were its used as an aditive to maintain nutritional and sensory quality (7-9).

Different trials that involve the use of organic solvents and other techniques kinder to the environment have been used for the extraction and quantification of this compound. This way different systems such as liquid–liquid extractions, solid phase extractions or supercritical fluid extractions, can be used (10). Liquid–liquid extractions are most common and many optimising variables can be found through the variation of the solvents and its proportions, for example Ordóñez-Santos & Vázquez-Riascos(11) recommend the combination of hexane/acetone/ethanol (50:25:25 v/v), acetate/hexane or acetone/hexane (10). However, these systems of extraction often present

problems such as: low extraction yields, long extraction times required, poor quality and final products with traces of organic solvents; all which added with the instability of the lycopene and the toxic and harmful potential of organic solvents to the environment, as well as the safety hazards and high energy costs involved (12), makes this extractions very complex (10,13). Thus, there is an increasing interest in exploiting and looking for alternative sources and methods of extraction for lycopene (2) and this is where an enzymatic cell wall lysis to improve the extraction yield of intracellular contents, represents an innovative method with great potential that has gain interest for the extraction of different kind of substances (8).

The potential use of enzymes lies not only on its capacity to catalyze reactions with exquisite specificity and selectivity, but also on its ability to function under mild processing conditions in aqueous solutions, and this makes them ideal assistants for extraccions, modifications or synthesis of complex biological compounds (12,14). In this way they have been evaluated in the extraction of capsaicinoids and carotenoids from chilli (Capsicum annuum L.) increasing the yields in 7% and 11% as an exclusively effect of the enzymes (15), in the extraction of oil of Guevina avellana mol and Silybum marianum seeds with improvements of 98% and 10.46% respectively (16,17), the extraction of lycopene from tomato tissues (Lycopersicum esculentum S.) upgrading the yield in 198% with cellulase and 224% with pectinase (8) and the extraction of phenolics from citrus peels were the highest recovery in the enzyme-assisted extraction process using CelluzymeMX was up to 65.5% (18). Although enzyme assisted extraction has been applied in different plant, there are no reports available on extraction of lycopene from guava pulp using this technique.

The aim of this study was to evaluate the extraction of lycopene from guava pulp assisted by the use of pectinolytic enzymes.

Materials and Methods

Chemicals

Pectinase (polygalacturonase) produced by the company Novozyme as Pectinex Ultra Clear®; produced from a combination of Aspergillus niger and aculeatus, with declared activity of 7900 PGNU/ml.

Hexane (96%), ethanol (99.9%) and acetone (99.8%) purchased from Merck; Glacial acetic acid (99.5%) from Panreac Qumica S.A.; Sodium acetate trihydrate (99.5%) and sodium hydroxide in pellet form (97%) from Carlo Erba Reagents.

Preparation of the samples

Indian Pink Guavas (Psidium guajava L.) with commercial maturity grade, obtained from the rural zone of Palmira, Colombia, were used as substrate. Batches of guava were blended for 3 minutes in a domestic blender with sodium acetate buffer (0.2M, pH 4.5), in a solid-liquid ratio of 1: 0.5 (w/v) and then divided into equal amounts in Erlenmeyers to provide samples for three repetitions and their controls, used later in the enzymatic treatment. The pH and temperature of the samples were controlled strictly, to guaranty that the activity of the enzyme employed was ideal. Thus, a pH meter HI 4221 (HANNA Instruments, Woonsocket, RI, USA) equipped with a thermocouple, was employed following the Colombian Technique Norm (NTC) No.4592.

Enzymatic treatment of the guava pulp

Different enzyme concentrations and incubation times were evaluated in the treatment of guava pulp. Thus, volumes of a pectinase enzyme solution (1:100) were added to samples of guava pulp previously mixed with buffer according to the item above and following the concentrations established in Table 1. The samples were incubated in a stirring plate for periods of 15, 30, 45 and 60 minutes at 28°C, after which all samples were submitted to a heat treatment at 70°C for 5 minutes to inactivate the enzyme. The obtained product, named enzymatically treated guava pulp (ETGP), was packed and stored under refrigeration for the subsequent extraction and quantification of lycopene. Triplicates and control samples were employed to evaluate each condition. Control samples, were subjected to the same conditions of temperature and stirring.

Table 1: The enzymatic treatments applied to the guava pulp

Treatment	Enzyme concentration (% v/w of substrate)	Substrate (g)	Volume of Buffer (mL)	Total Sample (g)	Vol. Enzyme added (mL)
1	0.010	166.67	83.33	250	1.667
2	0.014	166.67	83.33	250	2.250
3	0.017	166.67	83.33	250	2.833
4	0.020	166.67	83.33	250	3.333

Lycopene extraction and quantification

Lycopene was extracted following the procedure described by Ordóñez-Santos & Vázquez–Riascos (2010) (11). Briefly, 2.5 g of the sample (ETGP) was weighed in a 50 mL Erlenmeyer flask, and 25 mL of 2:1:1 hexane/acetone/ethanol was added. The flask was covered with aluminium foil, and then placed in ice water and shaken for 15 min in a stirring plate (Thermo Scientific Instruments LLC, Madison, WI, USA), after which 10 mL of distilled water was added and the sample was allowed to rest. A

sample of the organic (hexane) phase was then taken with a Pasteur pipette and lycopene was quantified as ug/g fresh material by measurement the absorbance of the extract at 503 nm against hexane in a Genesys 20 spectrophotometer (Thermo Scientific Instruments LLC, Madison, WI, USA) using quartz cells UV -6030.

Physicochemical analyses

The colour was measured in the surface (8 points) and the interior (5 points) of the fruit using aCR400 chroma meter (Konica Minolta Sensing INC, Japan) provided with a 2° standard observer, a D65 illuminant and a calibration pattern of Y(89.5) x(.3176) y(.3347), the colour parameters (L*, a*, b*) corresponding to the uniform colour space CIEL*a*b* were obtained directly from the apparatus. Also Titratable Acidity (TA), pH and total soluble solids content (SS) were determined to characterize the fruit following the Colombian Technique Norms (NTC) (19-21).

Statistical analysis

Results were expressed as means ± standard deviations. The experimental data conform to one-factor complete randomized blocks designs with three replicates, and were analyzed by one-way ANOVAs using fixed effects models and post hoc Tukey tests. All analyses were performed using SPSS for Windows v.18.

Results and discussion

The physicochemical properties of the guava fruit employed can be seen in Table 2. The pH and SS did not differ much from the values reported by other researchers who studied the same variety, such as Ordóñez - Santos & Vázquez - Riascos (11) , Rojas-Barquera & Narváez–Cuenca (1) and González et al., (22). The TA was consistent with the data registered by the first two sources previously indicated and lower than the reported by González et al., (22).

The colour of the pulp was dark red, similar results were found in the characterization made by González et al., (22).

Table 2: Physicochemical properties of guava fruit (means ± standard deviations)

Property	Value	
pH	4.19	± 0.09
Soluble Solids (°Brix)	9.24	± 1.18
Titratable Acidity[1]	0.45	± 0.10
$CIE_{L^*a^*b^*}$		
L*	55.17	± 2.03
a*	32.51	± 2.43
b*	17.51	± 1.41

1% of Citric Acid.

The effect of pectinase concentration and incubation time on the enzymatic extraction of lycopene from guava pulp is shown in Figures 1 to 4.

Pectinase at 0.01% v/w of substrate proved to be very effective in increasing the extraction yield of lycopene from guava pulp by 20.60% respect to the blank. An incubation time of 15 min was found to be optimum to degrade the cell wall (Fig. 1).

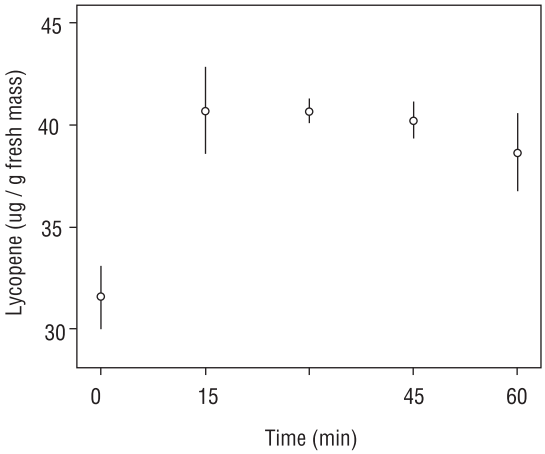

Figure 1: Incubation time on lycopene extraction from guava pulp, using pectinase at 0.01% v/w of substrate

Pretreatment of guava pulp with a concentration of pectinase of 0.0135 and 0.017% (v/w of substrate), allowed an increase in lycopene extraction of 29.80% respect to the blank, with an incubation time of 30 minutes (Figs. 2 and 3).

Pretreatment of the sample with enzyme at 0.02% (v/w of substrate), allowed an increase in lycopene extraction of 38.65%, after 45 minutes of incubation. Under these conditions, the lycopene extracted was 40.08 ug/g (Fig. 4).

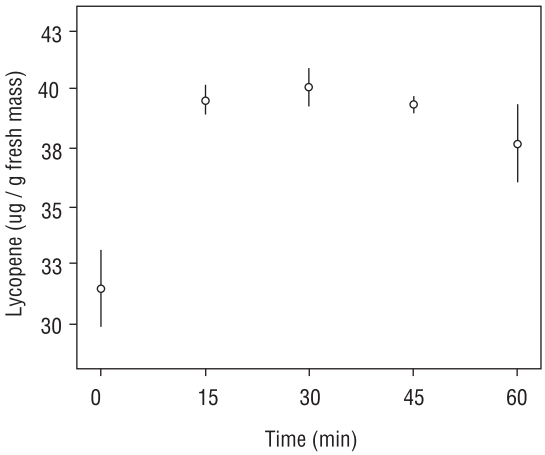

Figure 2: Incubation time on lycopene extraction from guava pulp, using pectinase at 0.0135% (v/w of substrate)

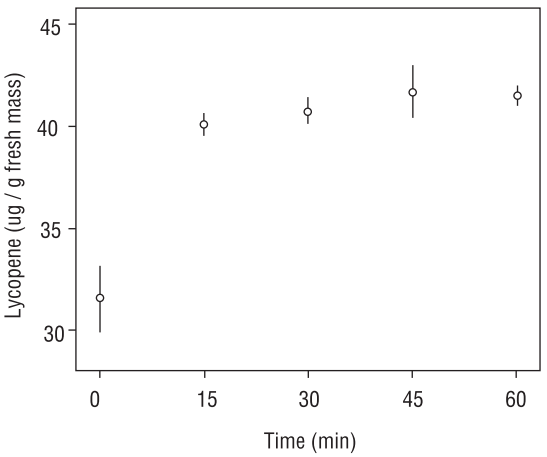

Figure 3: Incubation time on lycopene extraction from guava pulp, using pectinase at 0.0170% 0135% (v/w of substrate).

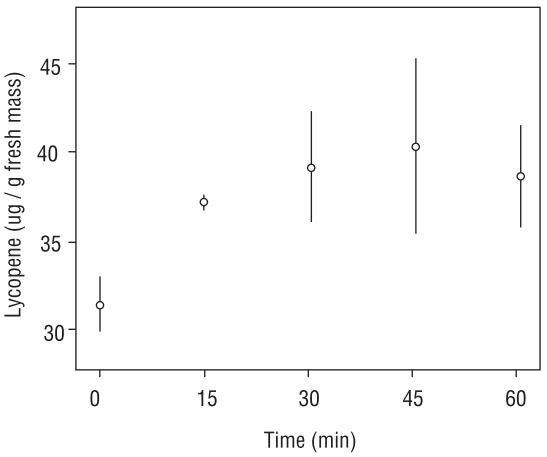

Figure 4: Incubation time on lycopene extraction from guava pulp, using pectinase at 0.020% (v/w of substrate).

Other studies involving pectinase enzyme as assistants in the extraction of lycopene compounds in which higher extraction yields were achieved, for example: Choudhari & Ananthanarayan (8) reports thatenzyme aided extraction of lycopene from whole tomatoes under optimised conditions resulted in an increase in the lycopene yield by 224%. Papaioannou & Karabelas (23) record extraction yields 20-25% in tomato peels, and Strati et al., (24) record extraction yields 650% in tomato waste. Respect of these differences is necessary to clarify that in all of these studies, differences in study samples, different enzyme activity, extraction methods and experimental conditions such as temperature and pH were used. Choudhari & Ananthanarayan (8) and Strati et al., (24) report that the extraction yields obtained by enzyme assisted extraction is associated with the pectinase being pectolytic and hemicellulolytic has the ability to disintegrate pectic compounds and pectin, the latter a polymer of 100-200-galacturonic acids, found in the middle lamella and primary walls. The degradation of structural components of tomato tissue, solvent molecules penetrates more easily the ruptured peel tissue and dissolves lycopene.

Conclusions

In this study it can be concluded that the guava pulp is an important source of lycopene, and shows that through the hydrolysis of the pectinolytic matrix of the plant tissue it's possible to improve conventional solvent extraction methodologies. The best result in the enzyme assisted extraction of lycopene was achieved with pectinase at 0.02% (v/w of substrate) of pectinase and 45 minutes of incubation time, with an extraction yield of 38.65%. Under these conditions, the concentration of lycopen extracted was 40.08 ug/g.

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Recibido: 25/07/2016.
Aprobado: 26/09/2017.