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Production Lycopene Dye São Caetano Melon (*Momordica charantia* L.) for Food Application

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Food industry use colorants are common because color appearance play an important role in the products acceptances. The aim of this work was to obtain lycopene dye from pulp São Caetano melon. Healthy and mature fruits were used, where pulp was removed from seed. After homogenization pulp, was used to extract lycopene, purification, identification, quantification, microencapsulation, characterization dye and its hygroscopic behavior application beverages. The analysis of São Caetano melon showed that it is an excellent source lycopene. Microscopy showed the encapsulation lycopene and the change in structure of pure maltodextrin encapsulated product. The main results show it was possible obtain powder dye with good hygroscopic characteristics strong red color, and easy apply drinks, with a result for similar color drinks already known in the market. São Caetano melon may be considered an option to the raw material for industrialization red dye used food industry.

1. Introduction

São Caetano melon belongs family Cucurbitaceae originated between south China and east India. In Brazil, species is common found on vegetable gardens, coffee plantations, etc. Mainly tropical regions, with very rare occurrence temperate areas (Coutinho et al., 2009). They present antibacterial, antifungal, antidiabetic, antiviral, antitumor, antileukemic, antioxidant properties etc. (Farias et al., 2009).
The seeds are enveloped in reddish and edible substance, they are bright red due high rate lycopene that can be used natural food coloring (Assubaie, 2004).
Lopes et al. (2007) reported that food industry the use colorants is common because color and appearance play important role consumer acceptance products. However, the ban some synthetic dyes some countries Europe and the United States has triggered growing concern over use these additives and several researches have been developed obtain dyes from natural products.
One of the main pigments found in liposoluble nature is lycopene, being responsible for red coloration in great variety fruits (Shami et al., 2004). Due to high oxidation rate of this compound during processing and storage it is necessary use media that preserve this component in elaboration and storage food products, this way searches are carried out to develop alternatives to this problem stability natural dyes.
The data use São Caetano melon seed in obtaining food dyes are scarce, however, some studies have shown high content lycopene in the pulp its seed. Therefore, is extremely important carry out studies make technological know feasible for industrialization São Caetano melon seed and to use them natural colorant for application various food products. The aim this research was obtain natural lycopene dye obtained from pulp São Caetano melon and it application on beverages.

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2. Material and Methods

Seeds of São Caetano melon (Momordica charantia L.) were used full yellow fruit peel coloration. The fruits were separated and removed the seeds manually, then pulp was removed from seed homogenized to obtain the lycopene extract.

2.1 Obtaining lycopene

2.1.1 Extraction, Purification, Nuclear Magnetic Resonance (NMR) and Quantification

For the extraction and homogenization of the pulp seed, were adding infusion earth and cooled acetone (4 °C) until paste mass. With the funnel the filtration was carried out, material retained in the filter was washed with acetone until removing all carotenoid. The obtained carotenoids were transferred to separation funnel containing petroleum ether, which was added distilled water removal acetone, this procedure was repeated until complete removal acetone (Rodriguez-Amaya, 2001).

Purification was performed open column chromatography containing magnesium oxide and infusion earth (1:1) using methodology with modifications described by (Rodriguez-Amaya, 2001). The purification procedure was performed with petroleum ether was removed and carotenoids were concentrated rotary evaporator, thereafter this concentrate was recovered with chloroform added the column mobile phase (chloroform) added thereafter. The separated red phase was collected, routed evaporator until total removal of chloroform. The purified concentrated lycopene extract was sent Nuclear Magnetic Resonance (NMR) also carried out quantification HPLC and sent to proceed the microencapsulation.

The concentrated lycopene was collected an aliquot and diluted Deuterated Cloformoio determination. ^{1}D and ^{2}D NMR spectra were obtained using Varian Mercury Plus BB (^{1}H: 300 MHz, ^{13}C 75 MHz) and Bruker Avance III HD (^{1}H: 500 MHz, ^{13}C 125 MHz) spectrometers. Tetramethylsilane (TMS) was used internal standard, and chemical shifts were recorded δ (ppm) values. TLC chromatography was performed on plates precoated with silica gel 60 and GF254 (10-40 μm).

After purification, lycopene extract was quantified HPLC. Lycopene determination was carried by chromatographic conditions described by Sant'Ana et al. (1998) with modifications. The devices utilized were: Chromaster liquid chromatograph (Hitachi, Kyoto, Japan) equipped with degasser, quaternary pump, autosampler set to 20 μL and diode array detector (DAD) set 470 nm. For chromatographic separation reverse phase C18 column (250 × 4.6 mm, 5 μm) was used. The mobile phase used Lycopene separation was composed methanol/acetonitrile/ethyl-acetate (80:10:10) rate 1.5 mL/min with running time 40 min. The quantification of lycopene was obtained using external standard curves. The sample and mobile phase were filtered through a 0.45 μm membrane (Millipore JBR61022 and HAWP04700, Bedford, MA).

2.2 Microencapsulation of lycopene

Purified lycopene was recovered with 96% cereal alcohol and homogenized with maltodextrin DE 20, ratio used was 8 % lycopene relative maltodextrin mass. The cereal alcohol was removed oven evaporation at 40°C until dry mass formed which then macerated obtain powder.

2.3 Stability of dyes

2.3.1 Adsorption isotherms

Determination of adsorption isotherms by static gravimetric method, described Wolf et al. (1985), using saturated solutions salts (CH3COOK, K2CO3, NaBr, SnCl2, KCL, BaCl2) temperature 21 ºC $\pm$ 2 ºC.

The adsorption isotherms were determined in triplicate. For each cell were weighed approximately 0.05 g aluminum crucibles and exposed cells with solution saturated salts. The process was followed weighing until equilibrium was obtained. After equilibration water activity samples was determined, temperature 25 ºC. Afterwards, they air circulation determine moisture content.

The equilibrium moisture (X_{eq}) was calculated difference between mass that sample presented equilibrium and its dry mass Eq (1):

$$X_{eq} = \frac{m_{eq} - m_s}{m_s} \quad (1)$$

Where: X_{eq} = equilibrium moisture (b.s.); m_{eq} = mass of the sample at equilibrium (g); m_s = dry sample mass (g).

For mathematical adjustment experimental data the dye isotherms, mathematical models GAB, BET, Henderson and Oswin were used.
The quality fit different models was evaluated means best values obtained from explained variation (R) and relative mean deviation (E %), defined by Iglesias and Chirife (1976) Eq (2):

$$E\,\% = \frac{100}{n}\sum_{i-1}^{n}\frac{|Xeq_e - Xeq_p|}{Xeq_e} \quad (2)$$

Where: *E* % = relative mean error; Xeq_e = values obtained experimentally; Xeq_p = values predicted by the model; n = number of experimental data.

2.3.2 Hygroscopicity and Degree of Caking

The analyzes were determined from methodology 14a and 15a, described GEA Niro Research Laboratory (2003), in which consists exposing powder relative air humidity (RH) 79.5 %, where was absorbed through powdered sample until constant increase weight was achieved. Afterwards moisture analysis, subsequently, moisture the dry dye was sieved with standard conditions (1200 μ mesh sieve). What was left sieve was expressed degree agglutination, degree caking. The parameters used characterize hygroscopicity and Degree Caking powder were obtained according GEA Niro Research Laboratory (2003).

2.3.3 Microscopy

A scanning electron microscope JEOL LSMP 100 (Japan) from Laboratory Microscopy of COMCAP (Central Analytical Agropecuária - UEM), which evaluated structures of obtained microparticles.

2.4 Application of dye in beverages

Microencapsulated lycopene were applied two types beverages that were white in color. They used natural flavored yogurt soy juice apple flavor. For purpose color comparison was used industrialized products such as yogurt and soy juice both strawberry flavor.
For each 10 g beverage 0.2 g obtained lycopene dye. Then, color analysis was performed using CIE method L *, a *, b * using a CR 10 (Konica Minolta) colorimeter.

3. Results

3.1 Identification and quantification lycopene

Analyzing results shown data chromatographic is Table 1.

Table 1: ^{1}H NMR spectra (300, MHz), compound Lycopene.

	(all-*E*)-Lycopene[a]		AMCV1	
H	δ_H	multiplicity; *J*(Hz)	δ_H	multiplicity; *J*(Hz)
2 and 2’	5.11	m	5.11	M
3 and 3’	2.12	m	2.12	M
4 and 4’	2.12	m	2.12	M
6 and 6’	5.95	d (*J* = 11.0)	5.96	d (*J* = 10.0)
7 and 7’	6.49	dd (*J* = 15.0, 11.1)	6.49	dd (*J* = 15.2, 11.1)
8 and 8’	6.25	d (*J* = 15.0)	6.25	d (*J* = 15.0)
10 and 10’	6.18	d (*J* = 11.4)	6.18	d (11.5)
11 and 11’	6.64	dd (*J* = 14.9, 11.4)	6.63	dd (*J* = 5.0, 10.0)
12 and 12’	6.35	d (*J* = 14.9)	6.36	d (*J* = 15.0)
14 and 14’	6.24	m	6.25	m
15 and 15’	6.62	m	6.62	m
16 and 16’	1.69	s	1.69	s
17 and 17’	1.62	s	1.61	s
18 and 18’	1.82	s	1.82	s
19 and 19’	1.97	s	1.97	s
20 and 20’	1.97	s	1.97	s

Analyzing results shown data chromatographic (Table 1), can be verified presence Lycopene, with 80.12 % purity by reversed-phase HPLC. ^{1}D and ^{2}D NMR spectra analysis was compared with literature and confirmed presence (all-E)-lycopene[1-7]. The actual purity (15Z)-lycopene was then considered sufficiently high subjected structural characterization using NMR spectroscopy, value estimated in normal-phase HPLC analysis.

The purified pulp extract São Caetano melon seeds obtained after elution open chromatographic column presented 80.12 % lycopene. Pacheco et al. (2012) they detected 94.2 % lycopene also São Caetano melon, these values were higher than one found study, however, these changes can be observed since the methodologies used determination lycopene HPLC were distinct, and fruit itself when planted different localities can vary quantification constituents, beyond the time harvest and other factors pre and post -harvest. The pulp São Caetano melon presented constitution the value 0.05 mg lycopene g^{-1}. It is observed that this fruit excellent source lycopene, as well as the other rich fruits this carotenoid as can observed Corrêa et al. (2015) watermelon 0,04 mg g^{-1}, guava 0,05 mg g^{-1}, Nwaichi et al. (2015) guava and tomato, respectively, 0,05 mg g^{-1} and 0.03 mg g^{-1}. This way São Caetano melon highlights use raw material for production lycopene dye, result which food matrix that does not stand out food industry for production products such as juices, extracts, sweets etc.

3.2 Stability of encapsulated lycopene dye

3.2.1 Adsorption isotherms

The parameters models applied experimental data adsorption isotherms lycopene dye, as well as the values of explained variation (R) and relative mean deviations (E %) used evaluation criteria representation isotherms are found Table 2.

The presented parameters can demonstrate that all models are able predict adjustments lycopene dye powder obtained from raw material São Caetano melon. All models presented R range of 95% and E % below criteria recommended Aguerre et al. (1989), where E % less than 10 % Indicates reasonable representation models, Labuza et al. (1985), In which the representation isotherms considered extremely good E % less than 5 %.

The other parameters also show excellent adjustments and isotherm that tends an asymptote when in activity equal to 1.0, being confirmed value K near 1.

However, the Henderson model presented best values to represent isotherms lycopene dye.

Table 2. Results of adjustment of the experimental data of the adsorption isotherm at 25 °C for lycopene dye.

Molds	Parameters				
	Xm	C	K	R	E %
GAB	0.104	0.466	0.973	95.16	2.76
	Xm	C	n	R	E %
BET	0.08	0.638	167.47	95.14	2.79
	a	b		R	E %
HENDERSON	0.522	3.032	-	95.36	2.60
	a	b		R	E %
OSWIN	0.063	1.096	-	95.19	2.80

R: variation explained. E %: relative mean error. Xm: moisture content in the monolayer. C: constant related to the heat of sorption of the molecular layer. K: GAB constant related to multilayers. N: BET constant related to multilayers. A and b: adjustment parameters of the Henderson and Oswin models

3.2.2 Hygroscopicity and Degree of Caking

The values found for determination hygroscopic behavior and degree of caking are, respectively, 6.65 % and 69.93 % lycopene powder dye.

Observed that dye presents value that fits non-hygroscopic powder, however, very binder in conditions relative humidity air above 70 %. Non-hygroscopicity may be linked use maltodextrin dye encapsulating agent, since its use causes a decrease hygroscopicity powder in general.

Powder caking undesirable phenomenon, which initially consists transformation powder into a chipped solid and sticky material, resulting in decreased functionality, fluidity and loss quality. However, the dye had a very high caking index parameter table (Table 1) and its values represent a very binder powder. According to Aliakbarian et al. (2015) states that conditions drying and microencapsulation process can improve the dissolution conditions water and retain the desired compounds during drying.

3.2.3 Microstructure

Microstructure images maltodextrin-encapsulated lycopene dye extracted from pulp São Caetano melon can be seen in Figure 1.

The microstructure above images demonstrates that macerated product reduced the size some particles that were globular large A and B, but also possible verify that A, B and C they have encapsulated lycopene globules. In D image, it shows the microstructure maltodextrin without the presence lycopene. Comparing it with other figures with encapsulated lycopene, was verified change structure studied particles with sizes and appearance larger and varied, porous and wrinkled corpuscles. According Ferrari et al. (2012), blackberry particles with 15 % maltodextrin the particles showed more wilted and wrinkled, with greater spacing between them. Thus, this research also found particles with wrinkled structures, and maybe associated with the use of maltodextrin.

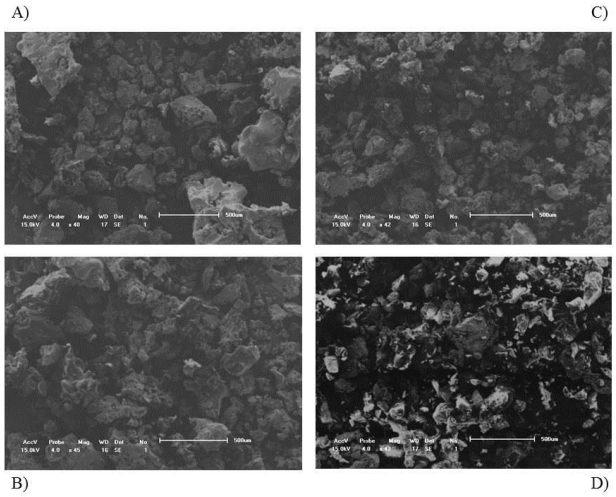

Figure 1. Microstructure images maltodextrin-encapsulated lycopene dye.
A) Encapsulated before maceration (pieces); B) Powder encapsulation before maceration*; C) Powder encapsulation after maceration; D) Maltodextrin DE 20.*

3.3 Applications of dye beverages

The values color determination are shown in Table 3.
The dye showed an intense red color, dark and opaque, being darker relation to that found for anthocyanin from dragon fruit encapsulated with maltodextrin where its a * ranged from 4.60 to 9.65 different drying times studied by Zaidel et al. (2015), thus demonstrating that lycopene is indeed excellent potential for application red dye. The natural yoghurt (NY), which was basis including dye, presented light color tending gray and pale. The standard strawberry yogurt (SYS) showed light red color (pink), dirty and pale color. The natural yoghurt added with lycopene dye (NYLD) presented light red (pink) coloration more intense landing than SYS.

Table 3. Mean values found for the color of lycopene powder dye and its application in beverages.

Beverages	Color parameters		
	*L	*a	*b
Dye	30.3 ± 0.06	30.83 ± 0.11	15.68 ± 0.23
NY	76.97 ± 0.65	-3,.7 ± 0.02	12.63 ± 0.03
SYS	52.33 ± 0.04	6.80 ± 0.02	7.30 ± 0.02
NYLD	54.73 ± 0.04	9.30 ± 0.07	10.43 ± 0.30
SJA	53.4 ± 0.12	-2.56 ± 0.03	4.97 ± 0.01
SJSS	46.06 ± 0.06	4.47 ± 0.08	2.73 ± 0.06
SJLD	42.4 ± 0.03	15 ± 0.10	6.94 ± 0.04

NY: Natural yogurt; SYS: Strawberry yogurt standard; NYLD: Natural yogurt with lycopene dye; JSA: Soybean juice apple flavor; SJSS: Soybean juice strawberry standard; SSMCL: Soybean juice flavored apple with lycopene dye.

The application dye juices was used apple juice soybean (SJA). The SSM showed light white color with tendency gray, dirty pale. The strawberry standard soybean juice (SJSS) showed light red coloration (pink) opaque color, dark light. The apple soya juice with lycopene dye (SSMCL) presented light red tint (pink) being tone darker than SPM and dark luminosity.
It turns out that colors obtained standard lycopene dye were within expected those already used food industry. Thus, by optimizing process obtaining this lycopene dye, São Caetano melon will become new source income agriculture.

4. Conclusions

The São Caetano melon was considered a lycopene source to be used as a raw material as a dye. The Maltodextrin is encapsulant which favors protection lycopene to use on colored beverages. The lycopene dye obtained from São Caetano melon was efficient providing the reddish color in those products that commonly use red dyes by food industry.

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