MICROENCAPSULATION OF HABANERO CHILLI (Capsicum chinense)
OLEORESIN IN β-CICLODEXTRIN AND ANTIOXIDANT ACTIVITY DURING
STORAGE

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Abstract: Habanero chili pepper (Capsicum chinense), is one of the most pungent
fruits in the world, and its production and demand have increased recently because of its
nutritional and medicinal applications. However, stability of its antioxidant components
is an important item to consider. Habanero chili oleoresin was microencapsulated in β-
cyclodextrin using a precipitation method at the oleoresin to β-cyclodextrin weight
ratios of 20:80 and 30:70. Antioxidant activity of the complex was evaluated during storage at different temperatures in the environment of aw’s from 0.115 to 0.967. Total
carotenoids content, scavenging activity of DPPH radical and total phenolic compounds
were measured spectrophotometrically.

Keywords: habanero chili oleoresin, β-cyclodextrin, molecular inclusion, antioxidant
activity

INTRODUCTION
Chilli peppers (Capsicum ss. vv.) are known and highly valued in all over the world due to their
colour and sensory attributes, basically, pungency, aroma and flavor. In Mexico, chilli Peppers
(Capsicum spp.) represent a culinary and cultural symbol, and as in many countries, an unforgettable
ingredient in lots of traditional dishes. (Singh et al., 2009; Pino et al., 2007; Topuz & Ozdemir, 2007).
Peppers (Capsicum spp.) belong to the Solanaceae family, native to Central and South America and are
cultivated also as a source of nutrients such carbohydrates, sugars, calcium, magnesium, β-
carotene, ascorbic acid, tocopherols, vitamins as C, E and A, and numerous non-nutritive bioactive
compounds known as “phytochemicals”, as phenolic compounds, that in the same way that carotenoids and
capsaicinoids have important antioxidant activity in nature (Singh et al., 2009; Antonius et al.,
2006).
The strong pungency and spicy flavor of the “hot” chilli peppers, has been attributed to a family of
compounds called “capsaicinoids”, always present - and only present-, in the fruits of the Capsicum
genus varieties in different amount, varying significantly from one to another. These are
interesting compounds that beyond their roles as flavor ingredients have also medical, toxicological
and therapeutic implications, in addition to their widespread use as a neuropharmacological tool, due
t heir effective action in the treatment of painful medical conditions. Indeed, capsaicinoids have been
reported as having antioxidant effect and antibacterial action on certain groups of bacteria
(Pino et al., 2007; Kurian & Starks, 2002).
Habanero chilli pepper (Capsicum chinense) is the most pungent pepper in México and almost the
hottest chilli pepper in the world. The more pungent, the highest content of capsaicin (Pino et al., 2007;
Antonius et al., 2006).
Natural capsicain is available in liquid form as capsicum oleoresin, oleic concentrates that contain
natural antioxidants of the corresponding spice, that unfortunately pose a number of problems in
handling and use, because of their sensitivity to light, heat and oxygen, and hence have a short
storage life if not stored properly (Meunier et al., 2007; Rosa et al., 2002).
Microencapsulation seems to be useful to solve these problems, specially carried out by the method of
molecular inclusion complexation, which occurs at the molecular level, whereby individual molecules of
food or flavor ingredients are partly or entirely trapped or included within the hydrophobic cavities
present in individual molecules of carrier. The most known carriers of this type are the cyclodextrines, and
β-cyclodextrine has been known as the most common and suitable cyclodextrin for
microencapsulation; it’s the most accessible, the lowest-priced and thus, probably the best studied
cyclodextrin in humans. Indeed, β-CD provides an effective protection for every single flavor
constituents present in a multicomponent food system (Cho & Park, 2009; Ayala-Zavala et al.,
2008; Challa et al., 2005; Martin del Valle, 2004).
So, the purpose of this study was to evaluate the stability and antioxidant properties over time, of the HCO-BCD molecular inclusion complex.

MATERIALS AND METHODS
Pure habanero chilli oleoresin (HCO) was bought to AMCO de México, S.A., and β-cyclodextrin (BCD) was bought to Sigma-Aldrich; acetone HPLC grade, ethanol, distilled water.

Complexation Process
A precipitation method (Bhandari et al., 1998) was used to prepare the HCO-BCD complex. Fifty grams (+/- 0.01) of BCD was dissolved in 500 mL of an ethanol to water (1:2) mixture maintained at 55°C (+/- 2°C) on a hot plate. A predetermined quantity of HCO dissolved in acetone was then slowly added to the warm BCD solution, stirring continuously (magnetic stirrer) and holding the temperature at 55 °C (+/- 2 °C). The heating was stopped following this addition, and the resultant mixture was covered and stirred for 4 h more. The final solution was refrigerated overnight at 4 °C. The precipitated BCD-HCO complex was recovered by filtration, and then dried in a convection oven at 50 °C for 24 h. The powder was then removed from the oven and allowed to air-dry at 25 °C for an additional 24 h in order for the powder to reach its equilibrium moisture content. The starting ratios of core material (HCO) to BCD were 30:70 and 20:80. Each starting ratio was prepared and investigated in triplicate. Subsequently, each of the investigated parameters were studied in triplicate for each prepared sample, and the results are reported as the means with standard deviation. Comparisons of the investigated parameters between treatments were done using the least significant difference (LSD) test at the 5% level (P < 0.05).

Storage at different research conditions
The microcapsules prepared, under each of the established proportions, were subjected to storage during the time which allowed determining its useful life (approximately 60 days), monitoring periodically their physical and antioxidant properties. Airtight glass bottles of approximately 750cm³ were used as desiccators, inside which were placed 10 individual samples of 0.5-0.7g in small aluminum pans, which were removed periodically for analysis. Each desiccator contained a different saturated salt slurry in the range of water activity a_w from 0.11 to 0.85 according to reported values (Rockland & Beuchat, 1986; Labuza et al., 1998). Three sets of nine desiccators each, were stored to constant temperatures of 25, 35 and 45 °C, respectively, during the whole research time.

Isotherms construction
Samples between 1 and 2 g of the HCO-BCD complex were introduced into glass desiccators containing P₂O₅ as a desiccant at room temperature for 2 weeks in order to get complete dryness. Two to three gram samples were then placed in desiccators containing saturated salt slurries in the range of water activity a_w from 0.11 to 0.96 according to reported values (Rockland & Beuchat, 1986; Labuza et al., 1998). Afterwards the equilibrium moisture content of the samples was determined at 25, 35 and 45°C by the gravimetric method (Lang et al., 1981), and adsorption isotherms constructed. Equilibrium was assumed when the difference between consecutive samples weighting was less than 1 mg/g solids. Determinations were made in triplicate.

Pigment extraction for UV-Visible Spectroscopy
All samples were obtained according to Hornero & Minguez (2001), with some adequations. 0.5 g of were extracted for 30 min and the extract was then filtered and transfered to a volumetric flask and made up to 50 mL.

Total carotenoids determination
Spectrophotometric determination of the red and yellow isochromatic carotenoids fractions in the HCO and the determination of total carotenoids, was carried out with a diode array spectrophotometer with UV-vis, Agilent brand, model 8453 and the absorbance data at 472 and 508 nm obtained were introduced in the developed formulas based on the Lambert & Beer Law for standards of known concentrations of both carotenoidal fractions: (Hornero & Minguez, 2001)

\[
C^R = \frac{A_{508} x 2144.0 - A_{472} x 403.3}{270.9} 
\]

\[
C^Y = \frac{A_{472} x 17244.3 - A_{508} x 2450.1}{270.9} 
\]

\[
C^T = C^R + C^Y 
\]

Where \(C^T\) is the total carotenoids value, \(C^R\) and \(C^Y\) are red and yellow carotenoidal fractions, respectively (all in μg/ml); \(A_{508}\) and \(A_{472}\) are the absorbances of each of the complex extract at the indicated wavelengths.

Total phenolic compounds
The amount of total phenolics was determined with the Folin-Ciocalteau reagent, according to Javanmardi et al. (2003), with a modification in the solvent, acetone HPLC was used instead of methanol. In a test tube to 100 µl of each sample, 5 mL of Folin Ciocalteau 1:10 (v: v), and 4 mL Na₂CO₃ 7.5% (w: v) were added and incubated at 45 ° C for 15 minutes. The absorbance of all samples was measured at 765 nm, using a Jenway-Genova spectrophotometer. The results were expressed as
milligrams of gallic acid equivalent per gram of dry weight (mg GAE / g dw).

Scavenging activity of DPPH radical

The antiradical capacity of the HCO-BCD molecular inclusion complex was estimated according to Duan et al., (2007), with some modifications. 100μL of the HCO-BCD complex extract was mixed with 2.9 mL of 0.1mM DPPH-acetone solution. After the solution was incubated for 30 min at 25 °C in the dark, the decrease of absorbance at 517 nm was measured using a Jenway-Genova spectrophotometer. Control contained acetone HPLC instead of the antioxidant solution while blanks contained acetone HPLC instead of DPPH solution. The inhibition of DPPH radicals by samples was calculated according to the following equation:

\[ \% \text{DPPH}_{SA} = 1 - \left( \frac{(A_M - A_B)}{A_C} \right) \times 100 \] (4)

where DPPH_{SA} is the scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl radical; \(A_M\), \(A_B\) and \(A_C\) are the absorbance of the complex extract, blank and control solutions, respectively.

Color determination

Color TEC PCM colorimeter was used to determine the changes with storage time of the L*, a* and b* color parameters in the CIELab scale (Mehmet et al., 2003).

RESULTS AND DISCUSSION

Microencapsulation

The weight of the HCO-BCD complex recovered was less than the amount of the materials originally used, either for 30:70 or 20:80 ratio of core material (HCO) to β-CD, but there was no significant differences (P>0.05) between preparation yields, that were around 89%, in both cases. This result may suggest that the maximum inclusion of β-CD with HCO had been reached.

Moisture sorption isotherms

The moisture sorption of different ratios of HCO-β-CD at 35°C is shown in Fig. 1. A constant uptake was observed up to aw= 0.4, thereafter the moisture uptake was consistent to almost reach the 14% water content reported for β-CD (Ayala-Zavala et al., 2008), which could indicate that the HCO constituents are positioned just in the hydrophilic sites of the β-CD molecule, increasing the capacity of microcapsules to adsorb water. Thus, when water availability increases around the microcapsules, water starts to interact with the polar groups of the β-CD unbalancing the equilibrium and HCO constituents are displaced (Cuhna-Silva & Texeira Dias, 2004). It is observed that both HCO-βCD complexes preparations presented type II isotherms, which are characteristic of hydrophilic polymers having both surface adsorption and absorption in the solid phase (Zhang & Zografi, 2000).

![Fig. 1. Moisture adsorption isotherms for HCO-βCD complex, at two preparation ratios, at 35°C](image)

Total carotenoids content

Figures 2 and 3 show the main degradation conditions of carotenoids content in the HCO-βCD complex. As expected, at conditions shown in figures 2 and 3 it was observed an initial rapid degradation of encapsulated carotenoids, following a first order kinetics followed, in the most of the samples, of a second but much slower first order kinetic too (Rodríguez-Huezó et al., 2004; Desobry et al., 1999). The rapid oxidation might correspond to the degradation of the surface carotenoids and those in contact with a pore or a bubble in the matrix as was found by Desobry et al. (1999).

![Fig. 2. Main carotenoids degradation conditions at the 20:80 complexation ratio](image)
the ratio of 30:70 at aw=0.536. It may be assumed that although the higher content of HCO, at this temperature it has not already taken place the water mobility mechanisms that lets water act on the hydrophilic places on the β-CD molecule.

![Graph](image)

**Fig. 3. Main carotenoids degradation conditions at the 30:70 complexation ratio**

**Total phenolics**
There was no significant losses of phenolic compound in the most of the samples, and the reason is very simple: phenolic compounds that act as antioxidants in food can be inactivated as a result of processing, storage and handling, but these factors can induce the formation of new compounds with antioxidant properties that can maintain or even improve the original antioxidant activity (Manzocco et al., 1998).

**DPPH radical- scavenging activity**
The most of the samples of both HCO-βCD different ratio complexes presented during storage only slight variations around their original antioxidant activity measured by quenching DPPH radical. It can be assumed that the HCO entrapped in the β-CD molecule, while going through the oxidation of its polyphenolic compounds, leads to the formation of stable intermediates which can still exhibit strong antioxidant activity (Manzocco et al., 1998). Also, it may be that at low aw’s antioxidants compounds are protected due its presence on the β-CD molecule, and at higher aw’s the water adsorbed facilitates reactions that produce new compounds with novel antioxidant activity.

**Color changes**
Color was determined by measuring parameters L*(lightness), a*(redness) and b*(yellowness) in the CIELab scale. Parameter a* was the most sensitive to the degradation of carotenoids during storage. No significant changes were observed in the most of the samples. However, at the 20:80 HCO-βCD ratio of complexation, the microcapsules lost the redness at 35 days of storage in aw=0.654 at 25°C, but at 45°C there was a sharp lost of color since the first week of storage at aw=0.103. In the other hand, at the 30:70 HCO-βCD ratio of complexation only the microcapsules stored at 25°C kept their color during the whole research time. At 35°C a slow fading of the complex color occurred at aw’s of 0.108, 0.215 and 0.318 in a period of about 5-6 weeks of storage; at 45°C where this effect appeared since the second week of storage, basically at aw’s from 0.103 to 0.599.

**CONCLUSIONS**
It was found that HCO-βCD complex can be successfully produced according to the precipitation method using β-CD. The mayor samples kept their antioxidant activity parameters and color around initial levels, showing no significant changes during 45 days. However samples stored at aw’s of 0.103 and 0.599 resulted in a sharp decline in the carotenoids content accompanied by a strong change in color from bright orange to very pale yellow since the second week of storage, being most noticeable in the lower ratio of preparation and in the higher temperature of storage; whereas at the higher ratio and temperature, samples presented a slight loss of total phenolic compounds. This study contributes to improve the storage conditions of the HCO encapsulated with β-CD, either alone or included in any drug or food supplement.

**NOMENCLATURE**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>β-CD</td>
<td>β-cyclodextrin</td>
</tr>
<tr>
<td>CD’s</td>
<td>cyclodextrins</td>
</tr>
<tr>
<td>HCO</td>
<td>habanero chili oleoresin</td>
</tr>
<tr>
<td>aw</td>
<td>water activity</td>
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