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Study of Ochratoxin: A complexation in coffee

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ABSTRACT

To study the complexation of ochratoxin-A (OTA) by coffee in a real environment (in coffee-drink) and with some coffee constituents able to inter act with the OTA as caffeine, chlorogenic acid and arginine. The coffee drink was prepared according to the "filter"method. OTA complexation tests were done at 23°C and at 90-100°C using test tube and filter-paper. OTA was extracted with a methanol / sodium bicarbonate mixture at 3%, purified on the immunoaffinity column "ochraprep" and quantified by HPLC with a fluorimetric detection. The results give a high rate of OTA complexation by the constituents of the hot coffee-drink was obtained. It depends on the volume of the coffee drink and the chemical compound. When hot, the rate of complexation is about 100% with 40 ml of coffee-drinking. After 20 min of heating at 200 ° C, 86.6% of pure OTA were degraded against 12.4% of disappearance of the OTA due to the complexation by caffeine, 11.6% due to arginine and 6% due to chlorogenic acid. However, the products of complexation or degradation are not detectable by fluorimetry.

Keywords: Complexation of OTA, drink coffee, caffeine, chlorogenic acid, arginine

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INTRODUCTION

Ochratoxin A is a mycotoxin which is predominantly produced by molds of *Aspergillus* and *Penicillium*. It has nephrotoxic, teratogenic and immunotoxic properties. Ochratoxin - A contaminates several food stuffs. Its presence in coffee was reported for the first time in 1974 [8]. Since then, many studies report concentrations ranging between 0.4 and 80µg.kg^{-1} [7, 9, 19, 11, 6, 16, 12, 25]. Contamination levels of OTA in coffee can vary by region to origin [12], climatic conditions and the post - harvest treatment used.

During technological treatments of coffee such as roasting and coffee-drinking preparation, some authors have noted a variation of the initial content of OTA. Much works has been published with conflicting results. Some report substantial reductions [27, 26, 3, 4, 16, 2, 17], others report only a slight reduction of OTA content [24, 20].

The coffee bean is rich in protein (10-15%), organic acids (mainly chlorogenic acids: 6-10%), alkaloids (including caffeine: 1 to 3%) [5]. Dissolved in water (drink coffee) or under the influence of temperature, some of these constituents of coffee are susceptible to inter act with the OTA and be degraded and/ or form complexes, and thus lead to reduce the initial content of OTA.

The objective of this work was to study the complexation of OTA by coffee in a real environment (in coffee-drink) and with some coffee constituents that were able to inter act with the OTA such as caffeine, chlorogenic acid and arginine.

MATERIALS AND METHODS

2.1 Biological Material
The experiments focus on green coffee (arabica and robusta), the roasted and ground coffee, and coffee drink.

2.2 Reagents
Reagents used in the determination of OTA were of analytical grade: methanol, sodium bicarbonate, phosphate buffer (PBS), acetonitrile, glacial acetic acid, distilled water, caffeine, chlorogenic acid, and arginine.

2.3 Equipment
The green coffee samples were roasted in a drum roaster BRZ type 2 (Probat-Werke) and were ground in a stone mill type Ditting. Laboratory thermostatic oven was used for hot tests. The immunoaffinity columns "Ochraprep" were used for the separation and concentration of OTA, and HPLC (LC - 10ADVP, Shimadzu) for OTA quantification.

2.4 Coffee roasting
Samples of arabica and robusta coffee were roasted in three standards: light, medium and dark. The light roast was done at a temperature of 150 °C for 8 min, while a medium roast was done at 150 °C for 10min and a dark roast at 150 °C for 13 min.

2.5 Determination of ochratoxin- A in coffee
The official method (method of the European Union) for the determination of ochratoxin A was based on the method published by Pittet et al [14] for soluble coffee, which is an adaptation of the method developed by Nakajima et al in 1990 [10].

OTA was extracted from 10g of coffee using a solvent (methanol / sodium bicarbonate at 3% V/M) for 30 min at room temperature. After filtration, 10 ml of the extract were diluted with 30 ml of PBS (phosphate buffer at pH 7.3) and were purified on the immunoaffinity column.

The extract was introduced into the immunoaffinity column where there was uptake of OTA by antigens. The column is then rinsed with PBS to release the foreign and non specific components. The toxin was eluted from the column with methanol. The mixture was then evaporated under a stream of nitrogen at 70 °C and the elute was collected in a beaker, and was dissolved in 1 ml of mobile phase and was quantified using HPLC.

The operating conditions are as follows: injection loop 100 ml, HPLC column C18 reverse phase, ODS 1 to 5 microns with precolumn thermostat at 35°C. The pressure is between 95 to 105 bars. Isocratic flow is 1mL/min. Detection is done by fluorimetry. The excitation wavelength is 333 nm and that of emission is 460 nm. The retention time of OTA is approximately 12.6 min.

2.6 Complexation of OTA in coffee drinks
The coffee drink was prepared according to the "filter" method. The concentration was 6 g of ground coffee for 100 ml of water at 90-100 °C.

Depending on the volume of coffee, corresponding concentrations of OTA were prepared to have similar final concentration of 17.5 $ng.ml^{-1}$ in all the mixtures. The volumes of coffee drink equal to 10, 20, 30 and 40 ml respectively were mixed with the OTA content of 35.2, 52.8, 70.4 and 88 $ng.ml^{-1}$ respectively.

The study of the OTA complexation was done on samples of arabica and robusta coffee. Contamination was done at room temperature (with a coffee at 23°C) or heated (90-100 °C) and the mixture was subjected to a mild agitation at room temperature for 30 min and the OTA was quantified.

It should be noted that during the hot test, OTA was added to coffee heated between 90-100C and the mixture was kept at this temperature for 10min.

2.7 Thermal stability of OTA: Filter-paper discs of 10 mm of diameter were impregnated with 10 µl of a pure standard solution of OTA at 200 $ng.ml^{-1}$. Series of five discs were placed on a cast iron (providing thermal inertia) and were heated under real conditions of roasting contaminated coffee (200°C for 20 min and 250°C for 10 min). The sampling times are 5, 10, 15 and 20 min for tests at 200°C and 2.5, 5, 7.5 and 10 min for tests at 250°C. A disc was removed at a specific time and was extracted in 1 ml of HPLC solvent for 20 min. The OTA was again measured.

For the tests in the hemolysis test tube, a sample of 1ml pure OTA was taken and evaporated to dryness under a current of nitrogen prior to heating. A similar procedure was undertaken using 1 ml of HPLC solvent prior to analysis.

2.8 Complexation of OTA by the chemical compounds: In a hemolysis test tube, 1 ml of pure OTA at 165.8 $ng.ml^{-1}$ was mixed with 1 ml of a solution of caffeine, chlorogenic acid or arginine at the same concentration (165.8 $ng.ml^{-1}$). The mixture was evaporated to dryness under nitrogen stream. The extract was heated to 150 °C for 0-25 min, and 200 °C for 0-20 min, with an interval of 5min. Tests were not done at 250 °C because of possible sublimation of caffeine or degradation of certain molecules at very high temperatures. The test tube from oven is allowed to get cold and the extract repeated with 1ml of HPLC solvent prior to analysis.

RESULTS AND DISCUSSION

3.1 Complexation of OTA in coffee drink

Before roasting and preparation of the coffee drink, the OTA was measured in green coffee to ensure that it is free from contamination by OTA.

3.1.1 Complexation at 23°C

Table 1 shows the OTA content in the coffee-drink after contamination and mild agitation.

Table 1: Complexation of OTA in coffee drink at 23°C

	Coffee volume (ml)			
	10	**20**	**30**	**40**
Pure OTA ($ng.ml^{-1}$)	35.2	52.8	70.4	88
Theorical OTA content in the mixture ($ng.ml^{-1}$)	17.5	17.5	17.5	17.5
Real OTA content after test ($ng.ml^{-1}$)	17.2	17.0	17.4	17.3

OTA content in the mixture shows that whatever the volume of coffee, almost all of the initial OTA (17.5 $ng.ml^{-1}$) was found when the contamination is done at 23°C.

OTA was not complexed by the constituents of coffee drink at 23°C.

3.1.2. OTA Complexation under heating condition

The trials included roasted arabica coffee with three degrees of roasting: light, medium and dark. The figure 1 and 2 show the degree of complexation of OTA by the arabica and robusta coffees, respectively, at 90-100 °C depending on the volume of coffee.

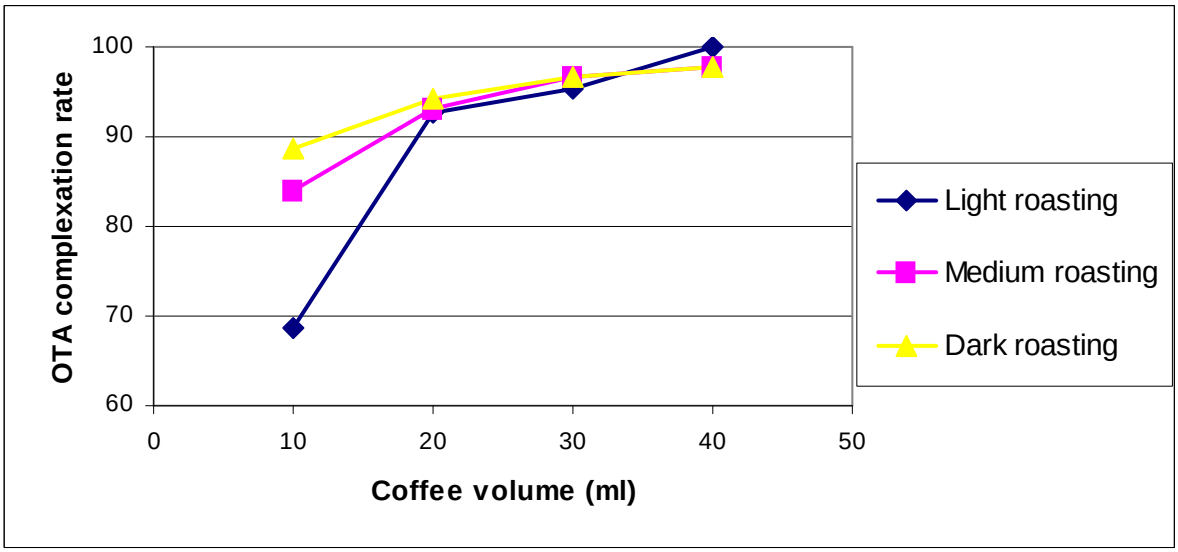

Figure 1: OTA complexation by the arabica coffee in hot condition

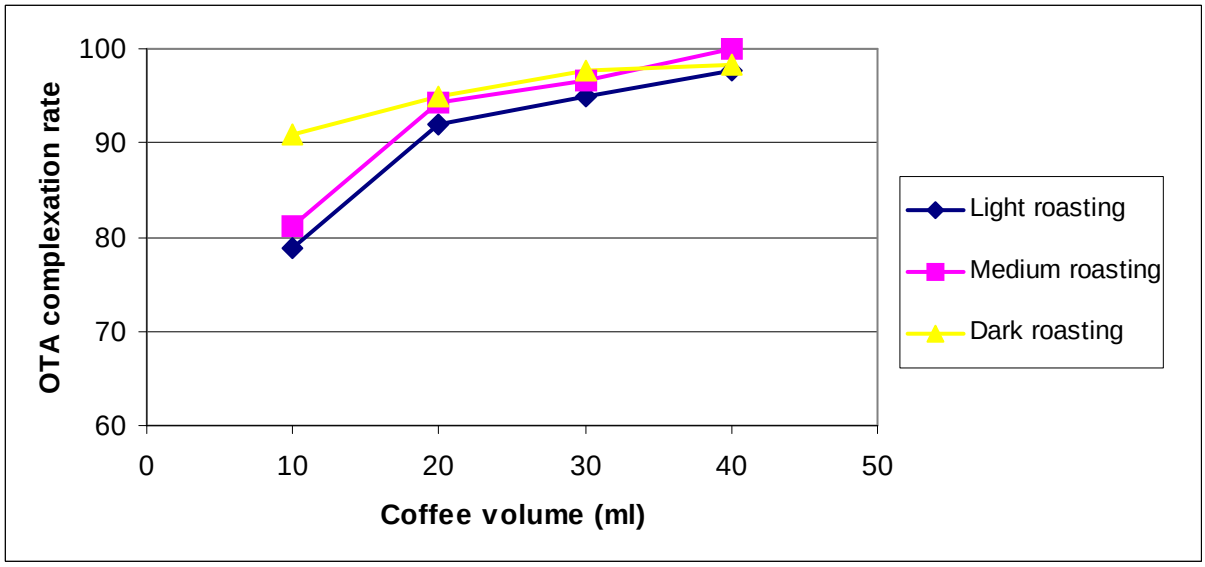

Figure 2: OTA Complexation by the robusta coffee in hot condition

When heated, components of Arabica and Robusta coffees are very reactive in presence of OTA whatever the degree of roasting. The complexation of OTA by these components was an increasing function of the volume of coffee used. It reaches a value of about 100% with 40 mL of coffee drink. For a given volume of coffee, the rate of OTA complexation was identical whatever the type of roasting except for relatively small volumes (10 ml) where there was a difference in the degree of OTA complexation. With such volume, the rate of complexation was depended on the type of coffee and roasting process. The minimum rate of OTA complexation was obtained with the light roast of Arabica coffee. It was 68.6% against 78.8%with Robusta. This difference was due to roasting. In the same condition, robusta coffee gets more roasted than Arabica.

Our results showed that thermal treatment has an impact on the content levels of ochratoxin A. Before 1988, it was thought that OTA was completely destroyed during roasting. Since then, many works were published with conflicting results. None reported high destruction after roasting [25, 4, 17], while others report only a slight reduction after coffee the roasting of naturally or artificially contaminated coffee [23, 19]. The rate of degradation of OTA during roasting was also a function of the initial content of OTA and the severity of the treatment applied [2, 15, 17]. During coffee roasting, there was the formation of brown compounds such as melanoidins due to Maillard reactions. These compounds, highly reactive, would be responsible for the OTA complexation observed. The intensity of the formation of these brown products is a function of the degree of roasting: this explains the low rate of complexation observed with light roasted coffee when the volume is small (10ml).

3.2. OTA Complexation in artificial milieu

Coffee is a very complex environment where there are several molecules that can interact with OTA. This is the case of caffeine, chlorogenic acid and arginine. Heating tests of these compounds in the presence of OTA was made in order to establish the degree of involvement of each other in the complexation of OTA observed during coffee roasting. Each product of interest was mixed with pure OTA and heated under real conditions of roasting contaminated coffee (200 ° C for 20 min and 250 ° C for 10 min). Before the OTA contamination with these chemicals, thermal stability of pure OTA was re-studied in the context of finding the product of complexation of OTA in hot condition described by Suarez-Quiroz et al [21].

3.2.1. Thermal stability of pure OTA

Tests on the thermal stability of OTA were made on several types of media: paper - filters (Whatman paper, paper glass fiber) and glass tubes hemolysis. Figures 3 and 4 show the kinetics of thermal degradation of pure OTA on filter papers and glass tube hemolysis respectively.

Results of the different kinetic obtained showed a linear degradation of OTA with increasing temperature. The kinetics of destruction of OTA has a single phase (1-order kinetics).

On the filter - paper (Figure 3), the slopes obtained were -0.03 min-1 at 200 °C and -0.10 min^{-1} at 250 °C. OTA concentrations obtained were better correlated with each other at 200 °C than at 250 °C. Similar results were found

by De Louise [2] and Suarez-Quiroz [21] as part of their research. They found slopes -0.02 min^{-1} at 200 °C and -0.09 min^{-1} at 250 °C.

It should be noted that the blank tests showed that the paper filters released cellulosic substances when heated. The chromatograms obtained have several spurious peaks that come with that of OTA. This is why the same tests were repeated in glass tubes in order to identify possible degradation products or OTA complexation products. The glass tubes are inert even at very high temperatures.

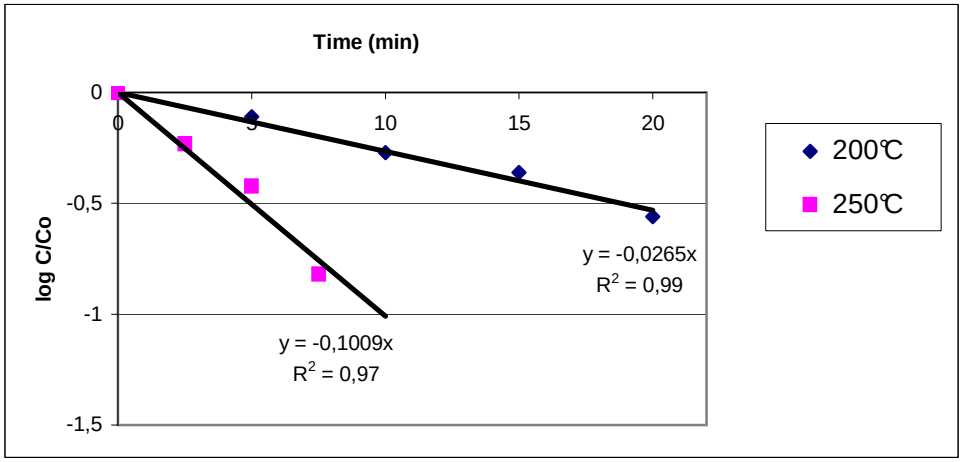

Figure 3: Kinetics of thermal degradation of pure OTA on paper - filter

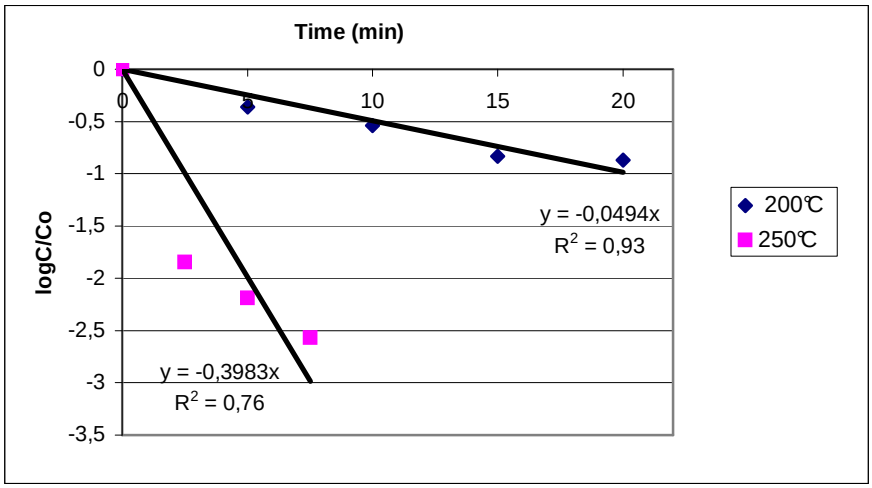

Figure 4: Kinetics of thermal degradation of pure OTA in the glass tube hemolysis

In the glass tube, the degradation kinetics of OTA was different from that obtained on paper (Figure 4). Degradation is faster than on the filter - paper because the K constants obtained was larger (-0.05 min-1 at 200°C and -0.40 min-1 at 250 °C). In the glass tube, the degradation was twice faster at 200°C and 4 times faster than 250 °C. The effect of the matrix was very important in the degradation of OTA under hot condition. The different chromatograms didn't show any new peak. This leads us to say that the degradation products of OTA under hot conditions were not detectable by fluorimetry. The additional peak described by Suarez-Quiroz et al [21] could come from the filter paper which released cellulose substances at high temperatures. For future tests, only the glass tubes will be used because of their thermal inertia.

3.2.2. OTA Complexation by chemical compounds

3.2.2.1 OTA Complexation at 150 ° C

Several samples, each containing 1 ml of a solution of pure OTA at 165.8 $ng.ml^{-1}$ and 1 ml of caffeine, chlorogenic acid or arginine at the same concentration (165.8 $ng.ml^{-1}$) were well mixed in a test tube. The mixture was evaporated to dry under a stream of nitrogen and heated for 5 to 25 min. Table 2 shows the OTA complexation after heating at 150 °C with a solution of caffeine, chlorogenic acid or arginine to 165.8 $ng.ml^{-1}$.

Table 2: Complexation of OTA by caffeine, chlorogenic acid and arginine at 150°C.

	0 min	5 min	10 min	15 min	20 min	25 min
OTA- caffeine ($ng.ml^{-1}$)	158.8	152.5	158.2	154.0	152.8	154.9
OTA- Chlorogenic acid	156.0	143.7	155.4	149.9	151.0	149.8
OTA-arginine	165.5	165.2	1554	151.7	160.4	155.3

In the presence of caffeine, chlorogenic acid or arginine, the concentration of OTA found remains almost unchanged, compared to the control unheated (0 min) regardless of the heating time.

At moderate temperature, there is no OTA complexation by caffeine, chlorogenic acid or arginine, whatever the duration of heating.

3.2.2 2. Complexation of OTA at 200 ° C

The same analyzes were done at 200 °C for 5 to 20 min. Figure 5 shows the complexation of OTA in the presence of caffeine, chlorogenic acid and arginine at 200 ° C.

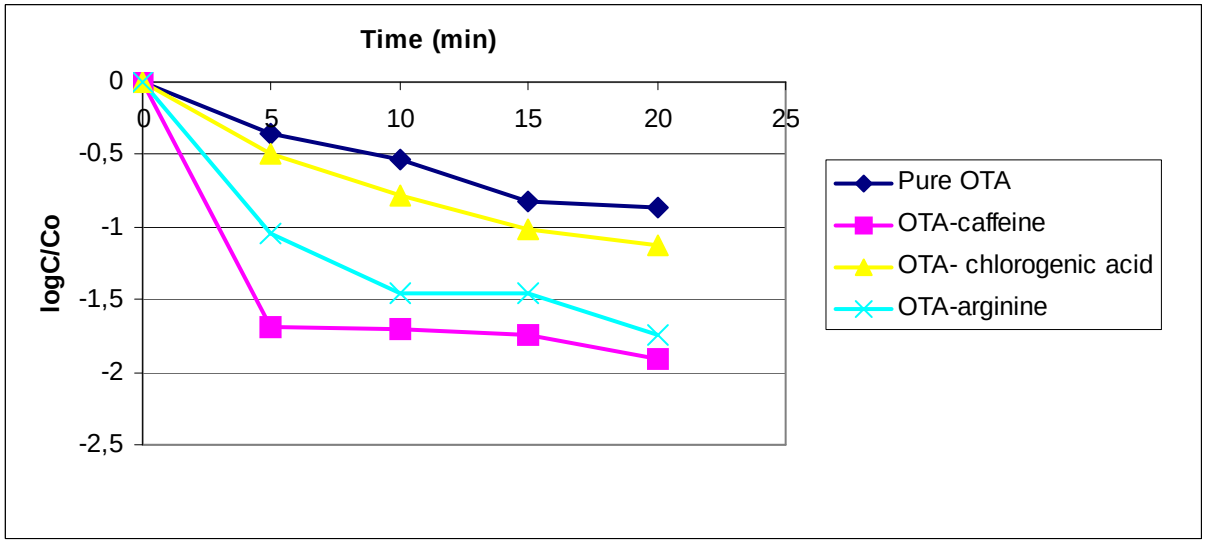

Figure 5: Complexation of OTA by caffeine, chlorogenic acid and arginine at 200 °C.

At 200 °C, OTA was deteriorated with time. Its content found in the mixture was decreased relative to that of pure OTA. This means that there is degradation and/or OTA complexation by caffeine, chlorogenic acid and arginine at 200 °C. The degree of reactivity was a function of the compound and the heating time. It is more pronounced with caffeine and arginine with which we observed a very rapid degradation of OTA followed by a slow down. As concerns the origin, the slopes or gradients were -0.0499 min^{-1} for pure OTA, against -0.0644 min^{-1} for chlorogenic acid, -0.1584 min^{-1} for arginine and -0.338 min^{-1} for caffeine.

After 20 min of heating at 200 °C, 86.6% of pure OTA was degraded by heat. Instead, when heated in a mixture under the same conditions, it is observed that 12.4% of the OTA disappear due to the complexation by caffeine, 11.6% due to arginine and 6% due to chlorogenic acid. At this temperature, chlorogenic acid was very unstable.

Tests of OTA complexation in an artificial environment have enabled the study of the state of OTA when heated in the presence of certain constituents of coffee such as caffeine, chlorogenic acid and arginine. A high rate of complexation was observed with caffeine and arginine. However, the products resulting from degradation or complexation remained unidentifiable by fluorimetry. It would be thus advisable to consider other detection methods to identify those products.

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