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Study of the effect of the green tea on the crystallization of brushite in the artificial urine with pH 6.5

MOKHBI ABDELWAHAB[1] and BAHRI FOUAD[2]

[1]Departement of Agronomy, [2]Department of Biology,
Faculty of Science and Sciences of Engineers, University of Mostaganem (Algeria).

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ABSTRACT

The tea plant, *Camellia sinensis* is cultivated for its sheets whose infusion and decoction allow the preparation of drink the most consumed in the world. Today, it is almost regarded as a drug. Certain crystalline species are evocative of a urinary supersaturation being able to rise of very different causes. Thus the brushite of the IVd type is induced by hypercalciuries with generally hyperphosphaturie and /or hyperparathyroïdie, disorder of the acidification, renal anatomical anomaly. The description of a possible inhibiting action of the tea on phosphocalcic crystallization with pH=6.5 can have very positive repercussions on the public health. That encouraged us to test *in vitro* the effect of its infusion and its decoction with three different concentrations on phosphocalcic crystallization starting from the artificial urine with pH=6.5.

In this objective, we thus developed a simple method of microphotography of which the duration of the experiment is 41 minutes. It makes it possible to appreciate the three phases of crystallization (nucleation, growth and aggregation). The kinetics of phosphocalcic crystallization with pH=6.5 was studied starting from the artificial urine. The crystallizable solutions of the urine artificial, and three concentrations different from the tea (C_1=4g.L^{-1}, C_2=16g.L^{-1} and C_3=32g.L^{-1}) prepared by infusion (t_1=5mn, t_2=10mn and t_3=15mn) and by decoction were maintained with 37C°, under constant agitation (150rpm) and with the darkness, to remain close to the physiological conditions. The follow-up of the kinetics of crystallization by optical microscope with polarized light and the spectral analysis by infra-red transformed of Fourier, reveal that, in the artificial urine without inhibitor, the formed crystals with pH=6.5 are brushite. The concentration (C_1=4g.L^{-1}) of the tea has a promoter effect on the three phases of crystallization. The addition of the C_2=16 g.L^{-1} concentration prepared by infusion during 15mn and decoction caused a reduction in the average size of the crystals and aggregates. The percentage of inhibition of the average size of the crystals and the aggregates of 63% and 84% for respectively C_2 is infused during 15 minutes (t_3) and by decoction. The inhibiting effect of the C_3 concentration on the crystallization of brushite with pH=6.5 is clearly highlighted in all the cases of figure. Its intensity varies according to the method of preparation. The percentage of inhibition of the average size of the crystals and the aggregates reaches 47%, 69%, 89% and 99% for respectively t_1, t_2, t_3 and decoction. Inhibition is proportional to times of infusion and it is maximum for the decoction.

Key words: Green tea, urine artificial, crystallization, brushite, inhibition.

INTRODUCTION

The tea plant, *Camellia sinensis*, is cultivated for its sheets whose infusion allows the preparation of drink the most consumed in the world[1-4]. *Camellia sinensis* is a shrub or a tree with persistent sheets. It belongs to the family of Theaceae[5]. Its height can reach 16 meters[6]. In culture, this one never exceeds the 3 meters, with a very dense ramification. *Camellia sinensis* is a plant which pushes, in India, in Sri Lanka, in the island of Java in China and in Japan[3]. Chinese traditional medicine prescribes this plant for various evils of the body in particular the headaches or those of a digestive nature[7-10]. Known to be stimulative and one désintoxificant of the organism[11]. It would act

also positively on the depressive state. Today, it is almost regarded as a drug thanks to these antioxydant capacities[12, 2, 13, 14], antiallergic[15, 16], antiatherosclerose[17] and antimicrobic[18, 19]. It would ensure a sufficient cardiovascular protection to prolong significantly the life[20]. By tradition, the Eastern and North-African populations prefer the green tea. Elsewhere, the other shapes of tea with sheets beforehand fermented or not are more or less taken such as the black, red, yellow teas or in the form of various commercial formulations. The tea contains volatile oils, vitamins, minerals, purins, polyphenols and particularly of the catechines[21, 22, 6]. The components bioactifs of the green tea were the subject of several researches[23, 2, 24, 25, 6, 20]. Its positive physiological effects combined with the activities antimutagenic, anticarcinogenic and antitumorgenic of the components polyphenolic of the green tea interests particularly the researchers[10, 11, 26]. The amino polyphenols and acids are the important factors in the determination of the quality of the green tea[27].

Certain crystalline species are evocative of a urinary supersaturation being able to rise of very different causes. Thus the brushite of the IVd type is induced by hypercalciuries with generally hyperphosphaturie or/and hyperparathyroïdie, disorder of the acidification, renal anatomical anomaly. The description of a possible inhibiting action of the tea on the crystallization of the brushite with pH=6.5 can have very positive repercussions on the public health. That encouraged us to test in vitro the effect of its infusion and its decoction with three different concentrations on phosphocalcic crystallization starting from the artificial urine with pH=6.5.

MATERIAL AND METHODS

Vegetable Material

The tea used in this experimentation comes from the trade. It is known under the name of green tea of China (reference 0071). It is the commercial reference most widespread on the Algerian market.

Prepared solutions

For our study, we chose the two methods of preparations used in Algeria, infusion, mode dominating the Algerian West and the decoction of which the use is largely appreciated in the Algerian South. Three concentrations different from the tea (C_1=8g.L^{-1}, C_2=16g.L^{-1} and C_3=32g.L^{-1}) prepared by infusion during t_1=5mn, t_2=10mn and t_3=15mn and by a 5 minutes decoction were tested.

Proportioning of metals, the Fluoride (F) and oxalate in the tea

The proportioning of following metals: Al^{3+}, Have, Ca^{2+}, Cd^{+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Mo, Na^{+}, Ni, Pb, Sr, Ti, V, Zn was made using an atomic apparatus of emission by plasma with inductive coupling (ICP-AE.S; Varian; Liberty Series II. Observation aviale). The potentiometric method was used for proportioning of the ion fluoride[28]. The determination of the concentration of the oxalic acid in the tea was carried out by using kits of diagnosis (SOBIODA SAS, ref.: 1310.001, France)[29].

Crystallization of the brushite with pH=6.5

The study was led by a method of microphotography inspired by that the Grases *et al.,*[30]. We developed it in our laboratory. It has the advantage on the practical level of reducing the 6 hours kinetics to 41 minutes. It makes it possible to appreciate the three phases of crystallization (nucleation, growth and aggregation).

Study of the crystallization of the brushite in absence of inhibitor

Preparation of the artificial urine

The artificial urine was prepared by mixing two equal volumes (100ml) of two solutions A and B whose composition is as follows:

- Solution A: 11.02 g.L^{-1} $Na_2SO_4.10H_2O$, 1.46 g.L^{-1} $MgSO_4.7H_2O$, 4.64 g.L^{-1} NH_4Cl, 12.13 g.L^{-1} KCl and 0.9g $CaCO_3$ dissolved in concentrated HCl.
- Solution B: 2.65 g.L^{-1} $NaH_2PO_4.2H_2O$, 18.82 g.L^{-1} $Na_2HPO_4.12H_2O$, 13.05 g.L^{-1} Na Cl, 1.0 g.L^{-1} $Na_3C_6H_5O_7.2H_2O$ and 0.05 g.L^{-1} $C_2O_4^{2-}$.

The precipitation of the solid phase of the brushite starting from the artificial urine was our investigation object with pH=6.5. 100 ml of the two solutions A and B are mixed under agitation and are maintained with 37°C in a bain-marie. The kinetics is followed during 41 minutes with regular intervals 3 minutes. The taken drop is examined

under polarizing microscope equipped with a numerical camera (Sony R model DKC-CM30). The size of the crystals and the aggregates is measured by an ocular micrometer.

Study of the crystallization of the brushite in the presence of the green tea

We took again the same method as in the first series to study the inhibiting effect of the green tea of China (reference 0071) on crystallization of the brushite with pH=6.5. The volume of the solution of the tea added to the solution B was 10 ml. We worked under parameters close to the physiological conditions of formations of calculations. The rate of inhibition is determined by the formula:

$I\ \% = [L - (T_{tea}/T_{si})]\ 100$ [31].

I%: Rate of inhibition;

T_{tea}: size of the crystals in the presence of the tea;

T_{si}: size of the crystals in absence of the tea.

RESULTS AND DISCUSSION

Biogenic salts, Fluoride (F^-) and oxalate

The proportioning of the biogenic salts by ICP revealed that the green tea of China (reference 0071) is rich in biogenic salts (table 1). The concentration of the biogenic salts is proportional to times of infusion and it is maximum for the decoction. There is also a linear relation between the concentration of tea (C_1, C_2 and C_3) and the rate of the biogenic salts. The concentration of potassium is higher than that of sodium. Except for the C_1 concentration, the magnesium contents are higher than those of calcium. The contents aluminum, iron, copper, manganese, zinc and nickel are rather important. The analyzed samples contain small quantities out of chromium, lead, arsenic, strontium and vanadium. We observed the absence of cadmium, tillium and molybdenum. Our results go in the same direction as those obtained by[20]. We noticed that the quantity of fluoride does not exceed the standards of WHO (1mg.L^{-1} in drinks) except for the C_3 concentration. Being a question of oxalate, it is present in the tea in sufficient quantity to present a risk of oxalocalcic lithogenesis. The green tea (reference 0071) is a rich food in oxalate especially prepared by infusion at long times and decoction and with high concentrations (C_2= 16g. L^{-1}, C_3= 32g.L^{-1}). Several works showed that the reduction of oxalate in the diet decreases the

Table 1: Results of the analyses of the metal elements, fluoride and oxalic acid of the green tea of China (reference 0071) out of mg.L^{-1}.

Mode of	[C]	Mg^{+2}	Ca^{+2}	Na^+	K^+	Al^{3+}	As	Cd	Cr^{+3}	Cu^{+2}	Fe^{+2}	Mn^{2+}	Mo	Ni	Pb^{+2}	Sr	Ti	Zn	V	F^-	oxalate
	C_1	8.83	8.95	2.33	78.87	1.43	0.11	0.00	0.001	0.10	0.55	3.46	0.00	0.03	0.005	0.016	0.00	0.45	0.012	0.38	23.43
t_1	C_2	23.22	21.24	3.31	241	5.77	0.16	0.00	0.005	0.19	0.95	13.03	0.00	0.17	0.006	0.025	0.00	0.77	0.061	0.97	93.75
	C_3	36.76	29.44	4.17	318.23	9.12	0.16	0.00	0.009	0.22	1.45	18.83	0.00	0.21	0.007	0.033	0.00	0.82	0.069	1.47	187.5
	C_1	9.33	10.12	3.12	85.74	1.74	0.12	0.00	0.001	0.10	0.62	3.74	0.00	0.05	0.005	0.019	0.00	0.50	0.012	0.42	27.75
t_2	C_2	25.58	23.16	3.75	251.96	5.98	0.17	0.00	0.006	0.20	1.12	13.89	0.00	0.19	0.007	0.028	0.00	0.79	0.064	1.02	106.25
Infusion	C_3	38.22	31.25	4.78	331.25	9.65	0.18	0.00	0.011	0.23	1.52	19.58	0.00	0.23	0.008	0.035	0.00	0.84	0.072	1.51	225
	C_1	10.22	12.56	3.85	92.56	1.98	0.13	0.00	0.002	0.11	0.85	4.36	0.00	0.08	0.006	0.021	0.00	0.54	0.013	0.46	30.25
t_3	C_2	27.33	25.63	4.12	255.36	6.23	0.17	0.00	0.007	0.21	1.28	14.25	0.00	0.21	0.007	0.029	0.00	0.81	0.071	1.11	118.5
	C_3	40.5	33.33	5.23	342.25	10.21	0.18	0.00	0.012	0.25	1.65	20.15	0.00	0.25	0.009	0.036	0.00	0.86	0.075	1.62	50.35
	C_1	12.5	14.36	3.97	102.33	2.12	0.13	0.00	0.002	0.11	0.92	5.68	0.00	0.12	0.006	0.022	0.00	0.61	0.014	0.53	34.52
Decoction	C_2	30.87	28.13	4.85	262.21	6.52	0.18	0.00	0.007	0.23	1.42	14.98	0.00	0.23	0.007	0.031	0.00	0.83	0.074	1.15	126
	C_3	42.33	36.25	5.79	355.56	10.56	0.19	0.00	0.014	0.26	1.72	21.35	0.00	0.28	0.009	0.038	0.00	0.91	0.078	1.66	265.63

Infusion : t_1=5mn, t_2=10mn, t_3=15mn; [C]: concentrations

concentration of urinary oxalate [32; 33; 2]. According to the results of the chemical analyses carried out, one can say that the green tea (reference 0071) contains a mixture of monoatomic inhibitors of crystallization phospho and oxalocalcic such as Mg^{2+}, F^{-}, Mn^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}, Cu^{2+} and promoters such as Ca^{2+} and oxalate[34].

Crystallization in absence of inhibitor
Morphology and size of the crystals

The analysis under the microscope made it possible to identify granulations of PACC (phosphate amorphous of calcium carbonates) and of the crystals in the form of rods and of punches slightly polarizing (Fig.1). The morphological identification of the crystals was carried out by comparing the crystals with those of the boards of identification given by[35] Jungero *et al.,* (1989).

The appearance of the granulations, the crystals and the aggregates intervenes respectively to 8, 20 and 23 minutes. (Fig. 2). At the end of 41 minutes, all the granulations are transformed into brushite (Fig. 2). The average size of the crystals (Cr) obtained is of 0.72µm, that of the aggregates is of 24µm (Fig. 2 and table 2).

Crystallization in the presence of the green tea
Morphology and size of the crystals and the aggregates with C_1=8g.L^{-1}

The granulations of PACC (amorphous carbonated calcium phosphate) appeared as from five minutes (5) of the kinetics in small quantities compared to without inhibitors (8 minutes). The morphology of the crystals obtained for the tea with weak concentration (C_1=8g.L^{-1}) prepared by infusion

Table 2: Evolution of the average size in µm of the crystals and aggregates of brushite

Times (min)	Cry	CV%	Agr	CV%
2	-		-	
5	-		-	
8	G		-	
11	G		-	
14	G+N		-	
17	G+N		-	
20	0.48+N	0	-	
23	0.72	<1	7.2	<1
26	0.72	<1	12	1.2
29	0.72	<1	16.8	1.1
32	0.72	<1	21.6	<1
35	0.72	<1	22.8	1.2
38	0.72	<1	24	<1
41	0.72	<1	24	<1

G: granulations of the PACC; N: nucleus; Cry: minimal average Size of crystals; Agr: maximal average size of the aggregates; **CV: coefficient of variation**.

Table 3: Evolution of the size in µm of the crystals and the aggregates of brushite without and with effect of 8g.L^{-1} of the tea prepared by infusion and decoction with pH=6.5.

Times (min)		2	5	8	11	14	17	20	23	26	29	32	35	38	41
SI	Cry	-	-	G	G	G	G	0,48	0,72	0,72	0,72	0,72	0,72	0,72	0,72
	Agr	-	-	-	-	-	-	-	7,2	12	16,8	21,6	22,8	24	24
t_1	Cry	-	G	0,25	0,5	0,75	0,75	0,75	1	1	1	1	1	1	1
	Agr	-	-	-	-	7,5	10	12,5	20	55	64,32	67	72	74	74
t_2	Cry	-	G	0,5	0,5	0,75	0,75	0,75	0,75	0,75	0,75	0,75	0,75	0,75	0,75
	Agr	-	-	-	-	7	9,6	10,75	16	45,9	49	52,25	61	61	61
t_3	Cry	-	G	G	0,25	0,25	0,5	0,5	0,5	0,75	0,75	0,75	0,75	0,75	0,75
	Agr	-	-	-	-	-	5	7,76	10	20	32	41	45	45	45
Dec	Cry	-	G	G	G	0,25	0,25	0,5	0,5	0,5	0,5	0,75	0,75	0,75	0,75
	Agr	-	-	-	-	-	-	5	7,76	14,26	20,26	29	34	34	34

G: granulations; Cry: minimal average Size of crystals; Agr: maximal average size of the aggregates.

(t_1, t_2, t_3) and by decoction did not change, the general shape of the crystals remains in the form of rods and of punches slightly polarizing, as for the case of the crystals in absence of inhibitors (Fig. 2). Nevertheless, the size seems higher (Table 3). The average size of the crystals and the aggregates is stabilized with 37.5µm, 30.88µm, 22.88µm and 17.38µm for respectively the three durations of infusion (t_1, t_2, t_3) and the decoction (Table 3).

Crystallization in the presence of the tea with 8g.L^{-1} earlier is compared with that without inhibitor. The formation of the crystals starts as from 8 minutes for two times of infusion t_1 and t_2. For the infusion t_3 time and the decoction times are respectively 11 and 14 minutes (Table 3). In absence of inhibitor (IF), the crystals appeared as from twenty minutes[20]. This concentration of the tea (C_1) supports nucleation. The aggregates appeared with 14 mn against 23 minutes in absence of inhibitor.

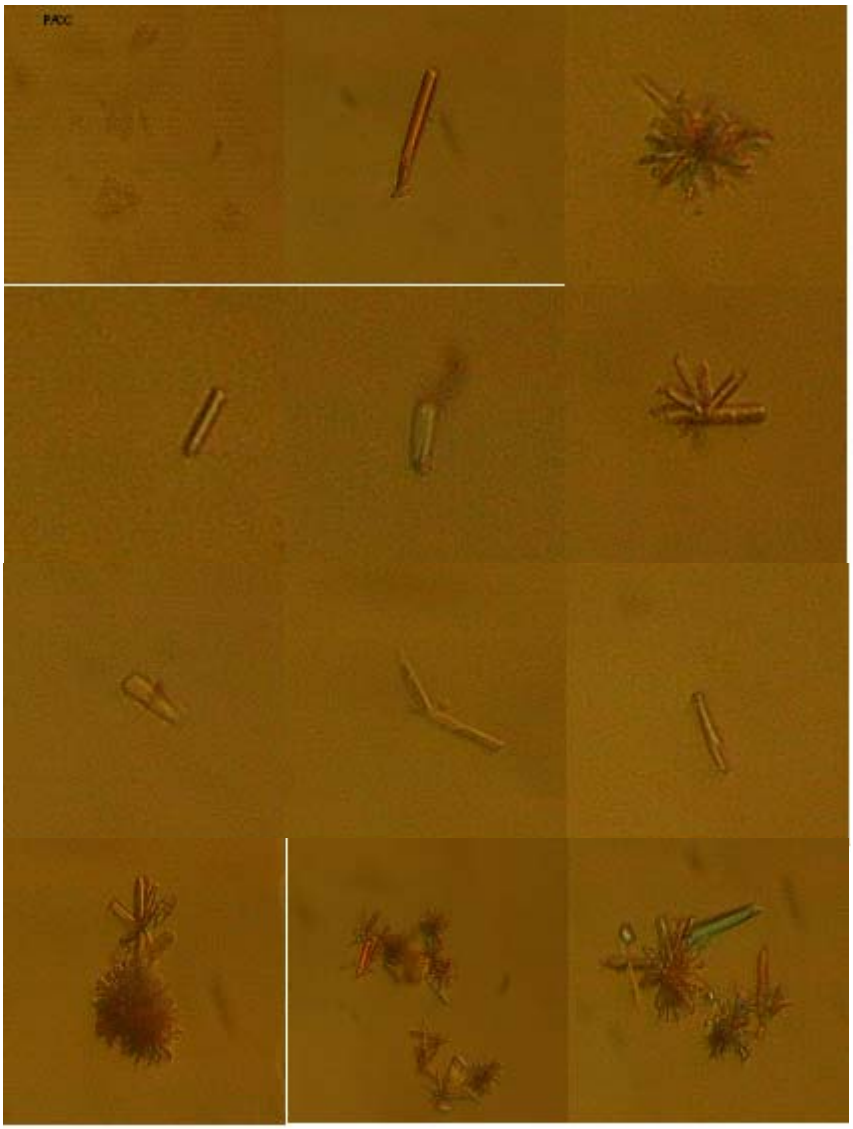

Fig. 1: morphology of the various crystals and aggregates of the brushite (acid dehydrate calcium phosphate) obtained in absence and in the presence of inhibitor with pH=6.5.

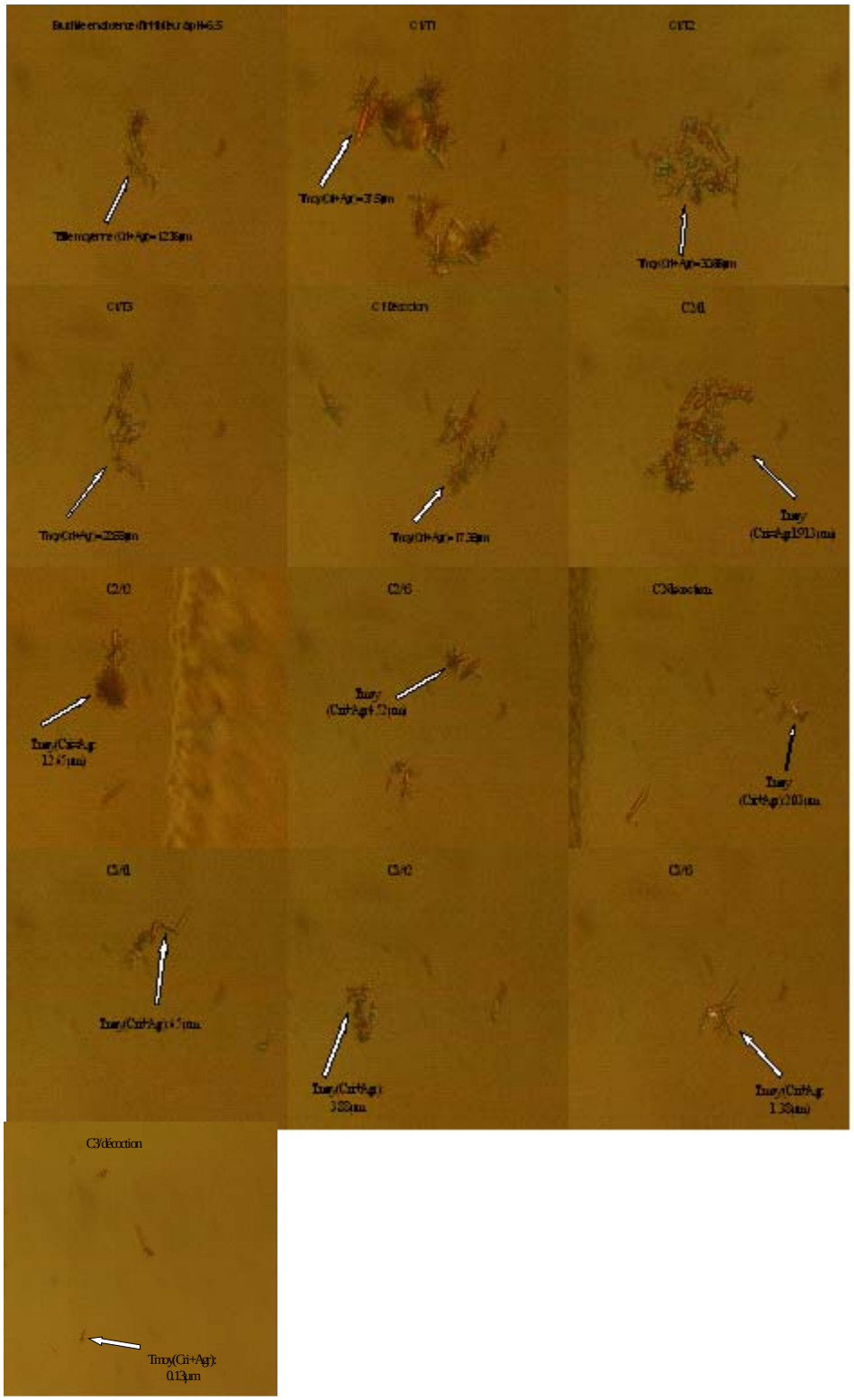

Fig. 2: Cut average crystals and aggregates of brushite obtained with the three concentrations of the tea (C_1, C_2, C_3) prepared by infusion (t_1, t_2, t_3) and by decoction

With this concentration, the tea supports nucleation, the size of the crystals and that of the aggregates.

Morphology and size of the crystals and the aggregates with C_2=16g.L^{-1}

It was observed during the study of the kinetics of crystallization, in the presence of the C_2 concentration of the tea prepared by infusion (t_1, t_2, t_3) and by decoction, by optical microscopy with polarizing light the formation of brushite as shown in the Fig. 2. The addition of this concentration did not cause a change of the general shape of the crystals. Nevertheless, the size of the crystals and the aggregates seems to decrease with the tea prepared by infusion (t_3) and decoction. On the other hand, the tea prepared by infusion during 5 and 10 minutes (t_1, t_2) supports crystallization by increasing the size of the crystals and the aggregates.

The evolution of the average size of the crystals and the aggregates according to time is given in table 4. The average size of the crystals and the aggregates is stabilized with 19.13µm, 12.65µm, 4.52µm and 2.03µm for respectively the three durations of infusion (t_1, t_2, t_3) and the decoction.

This concentration of the tea, prepared by infusion during 5 and 10 minutes, has a promoter effect on the average size of the crystals and the aggregates. On the other hand, the two remaining modes of preparation have an inhibiting effect.

Morphology and size of the crystals and the aggregates with C_3=32g.L^{-1}

The effect of this concentration of the tea prepared by infusion and decoction remains important. It was noticed during the study of the kinetics of crystallization, in the presence of the C_3 concentration of the tea prepared by infusion (t_1, t_2, t_3) and by decoction, by optical microscopy with polarizing light the formation of brushite as shown in the figure 2. The addition of this concentration did not cause a change of the general shape of the crystals. Nevertheless, the reduction in the size of the crystals and the aggregates is very important. We also observed that the preparation by decoction inhibits the formation of the aggregates of brushite (Fig. 2).

The evolution of the sizes of the crystals of brushite in the presence of this concentration prepared by decoction shows that up to 41 minutes of the kinetics, the crystals are still with the state of

Table 4: Evolution of the average size in µm of the crystals and the aggregates of brushite without and with effect of 16g.L^{-1} of the tea prepared by infusion and decoction with pH = 6.5.

Times (min)		2	5	8	11	14	17	20	23	26	29	32	35	38	41
SI	Cry	-	-	G	G	G	G	0,48	0,72	0,72	0,72	0,72	0,72	0,72	0,72
	Agr	-	-	-	-	-	-	-	7,2	12	16,8	21,6	22,8	24	24
t_1	Cry	-	G	G	G	0,25	0,5	0,5	0,5	0,5	0,75	0,75	0,75	0,75	0,75
	Agr	-	-	-	-	-	-	-	7	12,5	15	20	37,51	37,51	37,51
t_2	Cry	-	G	G	G	0,25	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3
	Agr	-	-	-	-	-	-	-	5	10	15	20	25	25	25
t_3	Cry	-	G	G	G	G	0,25	0,25	0,25	0,25	0,25	0,3	0,3	0,3	0,3
	Agr	-	-	-	-	-	-	-	-	-	-	-	1,76	6,24	8,74
Dec	Cry	-	G	G	G	G	G	0,2	0,25	0,25	0,25	0,28	0,28	0,28	0,28
	Agr	-	-	-	-	-	-	-	-	-	-	-	-	-	3,78

G: granulations; Cry: minimal average Size of crystals; Agr: maximal average size of the aggregates.

Table 5: Evolution of the average size in µm of the crystals and the aggregates of brushite without and with effect of 32g.L^{-1} of the tea prepared by infusion and decoction with pH 6.5

Times (min)		2	5	8	11	14	17	20	23	26	29	32	35	38	41
SI	Cry	-	-	G	G	G	G	0,48	0,72	0,72	0,72	0,72	0,72	0,72	0,72
	Ag	-	-	-	-	-	-	-	7,2	12	16,8	21,6	22,8	24	24
t_1	Cry	-	G	G	G	G	G	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
	Ag	-	-	-	-	-	-	-	-	6,26	7,75	11,26	12,5	12,5	12,5
t_2	Cry	-	G	G	G	G	G	G	0,25	0,25	0,25	0,25	0,25	0,25	0,25
	Ag	-	-	-	-	-	-	-	-	-	5	6,25	7,5	7,5	7,5
t_3	Cry	-	G	G	G	G	G	G	G	0,25	0,25	0,25	0,25	0,25	0,25
	Ag	-	-	-	-	-	-	-	-	-	-	-	1,25	2,5	2,5
Dec	Cry	-	G	G	G	G	G	G	G	G	G	0,25	0,25	0,25	0,25
	Ag	-	-	-	-	-	-	-	-	-	-	-	-	-	-

G: granulations; Cry: minimal average Size of crystals; Agr: maximal average size of the aggregates.

germination whereas the taking away in absence of inhibitor shows that there is already aggregation (table 5). Average size of the crystals and the aggregates and 6.5, 3.88, 1.38 and 0.13µm for respectively t_1, t_2, t_3 and decoction. The percentage of inhibition of the average size of the crystals and the aggregates is 47%, 69%, 89% and 99% for respectively t_1, t_2, t_3 and decoction.

CONCLUSION

The inhibition of the brushite, in the presence of 16 and 32g.L^{-1} of the tea, can be explained by the presence of the inhibitors such as Mg^{2+}, K^{+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, F^{-}, Fe^{3+}, Cr^{3+}, HCO_3^{-} and Al^{3+} in rather large quantity. The latter act by adsorption on the crystals of phosphate of calcium formed by blocking the sites of growth located on the crystalline faces [35, 34, 36]. The analysis of the metal elements present in the tea reveals that the following ions are proportional to the concentration of the tea: Mg^{2+}, K^{+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, F^{-}, Fe^{3+}, Cr^{3+}, $HCO_3^{-}$and Al^{3+}. These ions have an inhibiting effect on the crystalline growth of the crystals and the aggregates of calcium phosphates and oxalates[35].

The analysis of the ions magnesium Mg^{2+}dans the green tea gave rather important concentrations; to explain the inhibiting effect on the crystals of brushite. They are of 3.97 mg.L^{-1}, 6.13 mg.L^{-1} and 9.89 mg.L^{-1} for respectively C_1, C_2 and C_3 (table 1). The content of F^{-} is also important and proportional to the concentrations and infusion times of the tea. The average contents are of 0.45 mg.L^{-1}, 1.06 mg.L^{-1} and 1.57 mg.L^{-1} respectively for C_1, C_2 and C_3. The same correlations are noted with the other inhibiting ions such Al^{3+}, Zn^{2+} and Fe^{3+}.

Our results show that the C_2 concentration for the infusion t_3 time and the C_3 concentration for all the cases of figures exert an inhibiting effect contrary to the other weaker concentrations or less lengthily infused. Indeed, this report is coherent; inhibition is expressed for the treatments which conceal the highest contents of inhibiting ions (Mg^{2+}, F^{-}, Al^{3+}, Zn^{2+} and Fe^{3+}).

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