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RATE OF TRACE ELEMENTS (IRON, COPPER, ZINC AND MANGANESE) IN INGREDIENTS AND SAUCES CONSUMED IN MALARIA ENDEMIC ZONE IN ABIDJAN/COTE D'IVOIRE

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Eating habits provide mostly basic needs of the host, including micronutrients; also used by pathogenic organisms for their metabolism. Thus, knowledge of eating habits nutritional value in tropical infectious zone is essential to fight effectively against infections and avoid the loos in course of infections or food intake. In this study we investigated about micronutrients as iron, copper, manganese and zinc. A dietary survey lasted for 6 months leaded in malaria endemic zone in the east district of Abobo, Côte d'Ivoire). Samples of staple foods and their sauces are given by populations and the method of spectrometry atomic absorption used to evaluate iron, copper, manganese and zinc. Overall, sauces showed a predominantly iron compared to zinc, manganese and copper; the mean content of iron in ingredients and sauces such as fresh okra and eggplant was very high compared to other foods (P < 0.0001). "Gnagnan" sauce containing a higher quantity of iron was less consumed by populations. Considering the exogenous origin of these micronutrients, and the effect of their interaction, it would be suitable to evaluate their content in other foods and to study their effects in the host-parasite interaction to avoid the loss and fight effectively against infections.

Keywords: Trace elements, Dietary habits, Malaria-endemic zone, Cote d'Ivoire

INTRODUCTION

Eating habits provide majority of host needs, including micronutrients, also used by pathogenic organisms for their metabolism. To ensure a better nutritional status of the host and to fight against infections, sources of these micronutrients supply and their changes in the host-parasite interaction is essential (Herzog, 1992).

In Côte d'Ivoire, malaria remains one of the public health problems. Almost 43% of diagnoses are due to this disease (RCI/WHO, 2013). Children under five and pregnant women are more vulnerable (RCI/WHO, 2013; and WHO, 2014).

Although causes of malaria are certainly known, the interaction with the nutritional status of human host is difficult to establish (Caulfield *et al.*, 2004; and Osei et Hamer, 2008). In countries where holoendemic malaria coexists with malnutrition, they form a morbid and lethal combination, mostly among children under five years and pregnant women. The relationship between nutritional status and the worsening of endemic malaria remains subject of incessant investigations. Some studies show the negative impact of malnutrition on the health of patients; especially in children (Verhoef *et al.*, 2002); on the other hand, others reported the protective effect of malnutrition against malaria (Murray *et al.*, 1978; Anonyme, 2002; and Mitangala *et al.*, 2008 and 2012). Whatever may be said, malaria, like other infectious diseases such as measles, schistosomiasis and intestinal helminths, also causes significant nutritional

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problems (Verhoef *et al.*, 2002). The nutritional needs of the parasite for its growth and metabolism include essential nutrients from the host in which we have micronutrients (trace elements and vitamins) essential to the functioning of antioxidant enzymes and possibly the immune response (Osei et Hamer, 2008; and Mitangala *et al.*, 2008).

Although the immunomodulatory properties of zinc are known (Field *et al.*, 2002; and Roussel et Hininger-Favier, 2009), his interest in the treatment of malaria is variously interpreted (Anonyme, 2002; Field *et al.*, 2002; and Zeba *et al.*, 2008). Iron and copper are involved in the synthesis of hemoglobin; a prolonged deficiency of these micronutrients leads to anemia (Bonham *et al.*, 2002; and Harvey *et al.*, 2009). In malaria-infected areas, the interaction between iron and malaria worsening remains unsolved (Oppenheimer, 2001; WHO/UNICEF, 2006; and Zlotkin *et al.*, 2013). Zinc, copper, manganese and iron are coenzymes of superoxide dismutase (SOD), a modulation catalyzing enzyme of free radicals both in the host and in the *Plasmodium* (Müller, 2004). These trace elements are essential for both, host and pathogen to Regulate oxidative stress in malaria (Djossou, 1996; and Müller, 2004).

Since human host can't synthesize these nutrients their exchange with the parasite depend on exogenous supplies (Sandström, 2001). There may be therefore a competition between the host and the pathogen for the use of these micronutrients, what leads to the bioavailability problem of these micronutrients during the host-pathogen interaction.

This research dealt with the study of some micronutrients including iron, copper, manganese and zinc able to influence the growth of *Plasmodium falcip*arum, in eating habits of Abobo (Côte d'Ivoire) health district populations

MATERIALS AND METHODS

Study Area

This study was conducted among populations of Abobo health district in Abidjan (Côte d'Ivoire). It is limited in the north by the town of Anyama, in the east by Angré-Cocody, in the south by the commune of Williamsville and in the west by the Banco forest. It has a surface of about 9,000 hectares. The last census in 1998 (INS, 1998) put the population at 1,500,000 inhabitants with a density of 167 inhabitants per hectare. Abobo has a subtropical climate with a precarious socio-economic environment, which made it malaria endemic area with a high prevalence (Assoumou *et al.*, 2008; and M'boh *et al.*, 2010). We focused our attention on three health centers: South Banco-Anador, Kennedy-Clouetcha and Abobo-Baoule health formation.

Kind of the Study and Duration

That is a prospect study lasted for 6 months, to assess the rate iron, copper, manganese and zinc in foods consumed by populations and understands eventually the relationship with the sensitivity of malaria infection.

Biological Material

The biological material consisted of samples of staple foods involved in the cooking and sauces related.

Technical Equipment

The technical equipment consisted of a muffle furnace (570 °C) to obtain the ash, and a Atomic Absorption Spectrometer (AA Spert-5) for the determination of trace elements in food. Reagents were mainly composed of pure n-butanol, pure acids (nitric acid, trichloroacetic, hydrochloric, hydrofluoric), multi-element standard (Merck) and demineralized water.

Methods Food Survey

Data were collected using a frequency personal questionary of consumption (Subar FE and Thompson, 2013). Staple foods and sauces related were collected and analyzed in laboratory.

Evaluation of Dietary Habits

The food consumption frequency was expressed as percentage of consumers and many times consumed/week.

Sample Preparation

At the laboratory, samples of food (staple of foods and sauces) were pre-treated before the initial test. Foods were cleared of non-edible parts like bones, fishbones for sauces, peduncles for vegetable and stones for palm fruit, and then were weighed (W_s). Thereafter, wet samples were all pre-dried in oven (110 °C) till we got a constant weight before being crushed and weighed (w). The homogenates obtained were weighed (w_1) and put back in the dryer (103 °C) for 35-45 minutes, then weighed again (w_2). Free test samples were performed and we calculated averages.

Moisture and Dry Matter Determination

The rate of dry matter content was determined for 100 g of fresh matter; while the rate of moisture of each food was determined for 100 g of dry matter according to the following formulas:

Dessiccation without pre-drying: [1] %H =

$$H = \frac{me - m1}{me} \times 100$$

Dessiccation after pre-drying: [2]

$$\%H = \frac{\left(\frac{H}{100} \times me\right) + (me - m1)}{me} \times 100$$

%H1 (Moisture percentage of the sample); H2 (Moisture percentage obtained by desiccation of the pre-dried sample); me (Weight of sample); m_1 *(Weight after pre-drying).*

Determination of Ash

Ash or total minerals of each sample was determined by burning in muffle furnace (570 °C) for 3 hours in platinum crucibles, It was necessary to moisten once or twice with demineralised water in order to obtain a whitish ash (Pinta, 1973).

Determination of Fe, Zn, Cu, Mn in Ash

Iron, zinc, copper and manganese were determined by spectrophotometry atomic absorption with flame air/acetylene (SpertAA-5, Pattern®) with a threshold detection limit of 0.02 mg/L. Ashes were digested with diluted hydrochloric acid (5%) for Zn, Cu, Mn and hydrofluoric acid for Fe (Pinta, 1973). The multi-element standard solution with initial concentration of 1000 ppm (Merck) was extemporaneously diluted at 1/10 with hydrochloric acid (0.03 M) before being used for preparation of the standard range. Measurements of three samples were performed before determining averages.

Determination of Nutritional Intakes

Nutrients intakes were determined by calculating dry weights and minerals weight ingested per day by the following formulas:

[1] Wt = WI x P; *[2] Mt = Mt x Cx*

Wt (total weight of minerals); WI (Weight of ingested sauce); P (dry weight percentage of ingested sauce) Mt (total weight of minerals); Cx (mineral content of the used food).

Statistical Analysis of Data

Statistical analysis of data was performed using GraphPad Prism software. ANOVA was applied to the dietary survey results for the comparison of means (± standard error of the mean or SEM). Variations between trace elements concentrations means were analyzed using Welch's t-test. In order to determine the nutritional value of food, their contents in trace elements were compared with the Recommended Dietary Allowances. Differences were considered significant at $P < 0.05$.

RESULTS

Sauces Consumption Profile in the Population

Among about the ten sauces that normally are consumed with the staple foods, sauce prepared with fruits of *Solanum spp*. or "eggplant sauce" was the most consumed (62%). The improperly sauce named "light sauce", sauces made of *Arachis hypogea* fruit, locally called "peanut sauce", *Elaeis guineensis* fruit or palm seed, dried fruit of *Hibiscus spp*. or dry okra, were moderately consumed with respective consumption indices of 58.6%; 53.2%; 51.8% and 50.8%. Regarding the distribution of consumption, although consumption of eggplant sauce, "light" and peanuts, are weakly dispersed, the sauce made of dried okra had a very homogeneous distribution (CV = 7.1%). These results are shown in Figure 1.

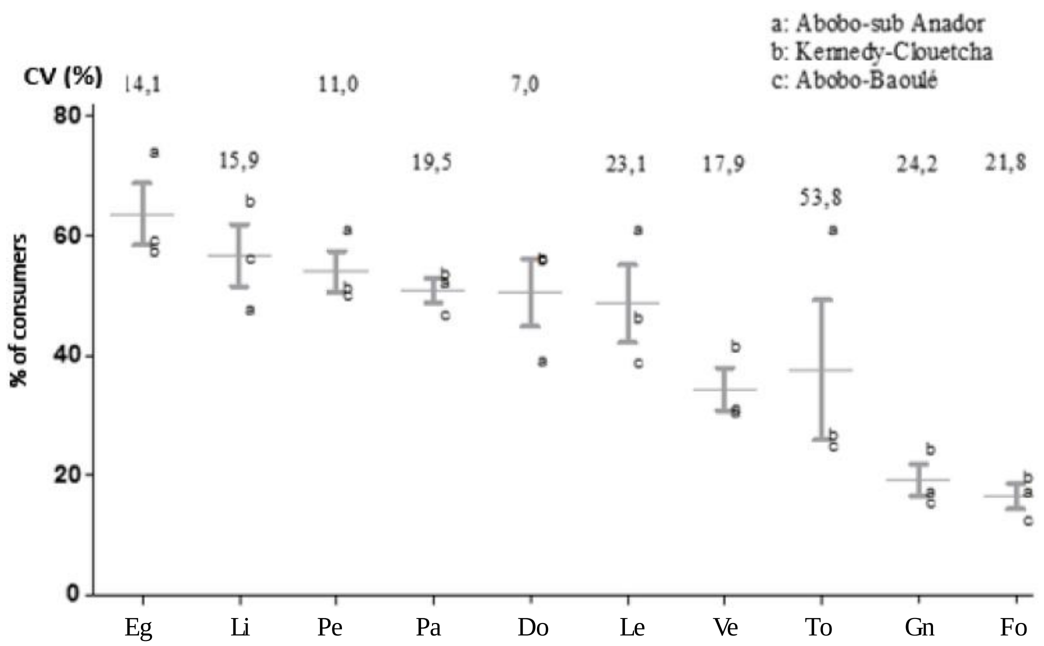
Moisture, Dry Matter and Total Minerals of Ingredients and Sauces

We noticed that among the fresh ingredients eaten (eggplant, fresh okra, "gnagnan" and palm seed), eggplant was the richest in water (91.03 ± 0.05%). We also observed, variations of water in sauces. Regarding the rate of total minerals, we observed on the whole that minerals percentages were higher for foods with low dry matter content; thus the sauce made of *Solanum indicu*m fresh fruit, "gnagnan" was the highest in minerals (16.28 ± 0.04%). Fruits of *Elaeis guineens*is (palm seed) contained lower mineral; in the sauce, the rate was 6.96 ± 0.05%. Significant differences ($P < 0.0001$) were observed between mean contents of these minerals. As expected, contents of total minerals increased in all sauces, compared to those of ingredients. However that increase was not dependent on the quantity of consumption sauces. The sauce made of "gnagnan" was the one that was slightly consumed (259 ± 0.1 g), but which provided a very significant ($P < 0.0001$) minerals (16.28 ± 0.04%). Sauces made of *Hibiscus spp* fruit or okra (fresh, dry) were also good providers of minerals, with respective rates of 16.12 ± 0.13% and 12.64 ± 0.04%. The results are shown in Table 1.

Content of iron, Zinc, Copper, Manganese of Ingredients and Sauces

We noted for ingredients and sauces that mean contents of iron in fresh okra and eggplant were significantly very high ($P < 0.0001$). For ingredients, the values are respectively

Figure 1: Weekly Distribution of Sauces Consumption in the Study Population



Note: *Eg: eggplant; Li: light; Pe: peanut; Pa: palmseed; Do: dried okra. Le: leaves; Ve: vegetables; To: tomato; Gn: gnagnan; Fo: fresh okra.*

Table 1: Moisture, Total Dry Matter and Minerals of Six Ingredients and Sauces

	Quantity (g)	Moisture (%)	Deshydrated Matter (%)	Total Minerals (%)
Staple Food				
Eggplant	100	91.03±0.05	8.97±0,01	7.09±0.01
Fresh okra	100	90.51±0.08	9.49±0,01	7.75±0.13
Gnagnan	100	83.08±0,04	16.92±0,01	6.87±0.04
Palm seed	100	32.59±0.13	67.41±0,01	1.31±0.28
Dried okra	100	10.09±0.01	89.91±0,42	9.43±0.43
peanut	100	05.06±0.02	94.94±3,76	2.58±0.15
Sauce				
Eggplant	299±0.5	83.41±0.75	16.59±0.11	10.60±0.02
Fresh okra	387±0.4	87.07±0.31	12.93±0.21	16.12±0.13[a]
Gnagnan	259±0.1	90.40±0.42	9.60±0.01	16.28±0.04[a]
Palm seed	276±0.0	84.85±1.05	15.15±0.03	06.96±0.05[b]
Dried okra	400±0.1	77.13±0.55	22.87±0.01	12.66±0.04
peanut	374±0.7	73.74±1.53	26.26±0.12	07.31±0.23[b]

Note: The amounts of sauces were determined without edible parties (edges and bones). The rates were determined for 100 g of initial material. (a, b) designes non-significant differences

0.99 ± 0.02 mg/g of fresh okra dry matter and 0.82 ± 0.01 mg/g of eggplant dry matter. Concerning sauces, values were very high ($P < 0.0001$). We recorded 1.82 ± 0.07 mg/g and 0.96 ± 0.01 mg/g respectively for fresh okra sauce and eggplant sauce. In addition, "Gnagnan" sauce was significantly high in iron. Manganese with mean contents of 0.06 ± 0.01 mg/g, 0.06 ± 0.02 mg/g and 0.05 ± 0.01 mg/g, respectively for dry okra, fresh okra and eggplant in ingredients, were significantly higher. Paradoxically, in sauces, these contents declined contrary to all expectations. These same remarks were observed for contents of zinc in fresh okra and in eggplant. We did not notice any significant variation of low cooper contents determined. All results are recorded in Tables 2 and 3.

Daily Nutritional Intake

Overall, the content of iron in sauces was significantly higher; the mean value consumed per day per person was 9.20 ± 1.65 mg/day. Zinc, manganese and especially copper were weakly consumed. Table 3 summarizes daily mean consumptions of the four trace elements determined within the six sauces evaluated for this purpose.

DISCUSSION

We noted significant variations in nutritional composition

Table 2: Trace Elements Content of Staple Food of the Study

Staple Food	Content of Trace Elements (mg/g)			
	Fe	Zn	Cu	Mn
Gnagnan	0,47 ± 0,02 [a]	0,02 ± 0,01 [c]	0,01 ± 0.01 [e]	0,03 ± 0,01 [f]
Dried Okra	0,38 ± 0,08 [a]	0,08 ± 0,02 [d]	0,01 ± 0,01 [e]	0,06 ± 0,01 [g]
Fresh Okra	0,99 ± 0,02 [b]	0,07 ± 0,01 [d]	0,02 ± 0,01 [e]	0,06 ± 0,02 [g]
Eggplant	0,82 ± 0,01 [b]	0,02 ± 0,01 [c]	0,02 ± 0,01 [e]	0,05 ± 0,01 [g]
Peanut	0,18 ± 0,02 [a]	0,03 ± 0,01 [c]	0,01 ± 0,01 [e]	0,02 ± 0,01 [f]
Palm Seed	0,33 ± 0,01 [a]	0,01 ± 0,01 [c]	0,02 ± 0,01 [e]	0,01 ± 0,01 [f]

Note: The difference is significant between different letters ($p < 0.05$).

Table 3: Trace Elements Content of Sauces of the Study

Sauce	Content of Trace Elements (mg/g) and Daily Intake (mg/day)			
	Fe	Zn	Cu	Mn
Gnagnan	1.94 ± 0.05 [a]	0.04 ± 0.01 [c]	0.02 ± 0.02 [e]	0.10 ± 0.01 [f]
Dried Okra	0.76 ± 0.02 [b]	0.08 ± 0.01 [d]	< 0.01 [e]	0.04 ± 0.01 [g]
Fresh Okra	3.64 ± 0.07 [a]	0.06 ± 0.01 [c]	0.02 ± 0.01 [e]	0.10 ± 0.01 [f]
Eggplant	1.92 ± 0.01 [a]	0.04 ± 0.01 [c]	0.02 ± 0.01 [e]	0.08 ± 0.01 [g]
Peanut	1.08 ± 0.04 [b]	0.12 ± 0.02 [d]	0.02 ± 0.02 [e]	0.06 ± 0.01 [g]
Palm Seed	0.88 ± 0.02 [b]	0.02 ± 0.01 [c]	0.02 ± 0.01 [e]	0.02 ± 0.01 [g]
AMJ (mg/j)	9.20 ± 1.65 [h]	0.52 ± 0.20 [i]	0.10 ± 0.02 [i]	0.46 ± 0.10 [i]

Note: The difference is significant between different letters ($p < 0.05$).

of foods as well as in ingredients in sauces. These variations are due to many factors, including the degree of maturity, sunlight, soil type, genetic factors and especially the water content of food (Herzog, 1992). Thus, mean water contents determined in this work are to be considered as point values from a range of possible contents; because of the factors listed above, and especially because of the post-harvest period of food before consumption or feeding practices in households. These practices differ from one household to the next; as it was observed with water content and dry matter content in sauces. Concerning minerals, we determined high iron content in ingredients. For these contents were strongly influenced by biological factors (Herzog, 1992) and analysis techniques (Pinta, 1973; and Herzog, 1992) it was not easy to comment on these variations. Nevertheless, the limited data available (Pinta, *et al.*, 1973; and Kouassi *et al.*, 2013) confirmed that vegetables contain more iron than other minerals of the study. The iron was also significantly consumed, but its daily mean intake (9.20 mg) would be slightly below the recommended mean for an adult to cover the daily needs (14 mg). Similarly, manganese, zinc and copper were slightly brought compared to their recommended intake. However, these results should be considered as a fraction of the exact intakes, as snacks between main meals and breakfast were not included in this work. This would have certainly influenced the determined values. However, since the dosed sauces (lunch and dinner) accounted for the bulk of the mean daily consumption, these results were used to assess the overall nutritional intake of food. These corroborate the first published reports of the study (Kolia *et al.*, 2014a and 2014b) and allowed us to note in general a deficiency of iron, zinc, manganese and copper intake of Abobo-east health district population.

The eggplant sauce which had a high value in iron was highly consumed by populations with others foods as "light sauce", *Arachis hypogea* "peanut sauce", *Elaeis guineensis* "palm seed" and dried fruit of *hibiscus spp*. "dried okra" fruit. Thus the frequently consumption of these food in high endemic zone of malaria would constitute a risk for the multiplication of parasite. In effect iron is necessary for parasite multiplication. In the treatment of malaria, correcting iron deciency is important, because malaria causes hemolysis and anemia. Supplementation in some cases, however, may actually aggravate infection, because the malaria parasite requires iron for its mutiplication in blood and thus may be less infective in the iron-decient person (Stroble, 2005).

The likely nutritional inadequacy intake determined in this work, that to say a predominantly iron in sauces compared to zinc, manganese and copper would be, the risk of parasite multiplication. In effect, an inadequate dietary intake leads to weight loss, lowered immunity, mucosal damage, invasion by pathogens, and impaired growth and development in children (Strobel, 2005). Zinc deciency reduces nonspecic immunity, including neutrophil and natural killer cell function and complement activity; reduces numbers of T and B lymphocytes; and suppresses delayed hypersensitivity, cytotoxic activity, and antibody production (Strobel, 2005). Being given the importance of zinc, copper and manganese for human body, a prolonged deficit by inadequate of food intake would lead to a nutritional deficiency that will absolutely affect immunity and increase the risk of morbidity and mortality related to endemic malaria (Strobel, 2005; and Katona et Katona-Apte, 2008). Therefore, the results of this study could justify the interest of a nutritional supplementation associated with malaria treatment as proposed by certain investigations (Zeba *et al.*, 2008; and Zlotkin *et al.*, 2013). It could help to compensate for the lack of food intake reported here, and strengthen the immune system. However, it is worth commenting. The essentiality of iron for parasite survival is unquestionable (Wander *et al.*, 2009); it serves among other things, as cofactor of SOD iron-dependent to regulate the excess of free radicals formed during malaria (Müller, 2004). His uncontrolled intake may promote malaria exacerbation (Oppenheimer, 2001; and WHO/UNICEF, 2006) or the emergence of a low parasitaemia sometimes controlled by the body. Thus, the deficit of its intake (9.20 mg/d) determined in this study can be described as minor risk which does not require absolutely supplementation during a treatment. For the more, the law food intake about zinc and copper noted in the study could have negative effects on the immune system (Roussel et Hininger-Favier, 2009); idem for manganese. Given these observations, and since the intake of these micronutrients is exclusively exogenous, it is desirable to extend the study to other foods in order to establish a nutritional profile that can serve as additional therapy. For the more the consumption of these foods must be checked to avoid effects of interaction of micronutrients which could limit their absorption thus resulting in deficiency (Abdel-Mageed et Oehme, 1990).

CONCLUSION

Considering the exogenous origin of these micronutrients, and the effect of their interaction, it would be suitable to evaluate their content in other foods and to study their effects in the host-parasite interaction to avoid the loss and fight effectively against infections.

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