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Effect of processing and preservation processes on microbiological quality of *Coptodon guineensis* and *Sarotherodon melanotheron* in South Benin

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Abstract

Fish is a very perishable foodstuff. The multiplication of the pathogenic germs is a problem of public health. The study aimed to evaluate the effect of processing and preservation processes on the microbiological quality of fish in south Benin. The study was carried out from June 2014 to December 2014. One hundred and twenty (120) samples of *Sarotherodon melanotheron* and *Coptodon guineensis* processed or not were used for microbiological analyzes. The samples were put in Stomacher bags at the embankment and in the kpota market, then stored under ice at 4°C and sent to the laboratory. At the end of the microbiological analyzes, the processing mode had no effect on the Total Mesophilic Aerobic Flora (TMAF), fecal coliforms and Sulfite-Reducing Anaerobes (SRA) load (P> 0.05). However, total coliforms loads of whole fish, pre-processed fish, and smoked fish were higher than that of fried fish. The TMAF, total coliforms, staphylococcal and SRA loads of *S. melanotheron* were not different from those of *C. guineensis*. On the other hand, *S. melanotheron* had a fecal coliforms load significantly higher than that of *C. guineensis* ($0.73x10^3$ *vs* $0.32x10^3$ CFU/g). The *Salmonella* percentage of *S. melanotheron* (26.67%) was identical (P>0.05) to that of *C. guineensis* (30%). The highest *salmonella* percentage was obtained in pre-processed samples (50%) and the lowest (P<0.05), were recorded in fried (10%) and smoked (10%) fish. Improving fish processing and preservation techniques will ensure a better quality of fish to the consumer.

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Introduction

In Benin, fish importance and its vital place in the diet are now widely recognized. Being a perishable food (ANSES, 2015), several methods of preservation and processing are used to guarantee its quality integrity before consumption. The processing methods are often of artisanal type (smoking, frying and salting-drying) and don't pay close attention to hygiene regulations for human health (Goueu, 2006; Abochi, 2010, Degnon *et al*., 2013; Chabi *et al*., 2014; Kpodékon *et al*., 2014). In addition, a poor processing of these fish and a poor preservation of these products can lead to germs proliferation which provokes food poisoning or intoxination.

These germs include the total flora, total coliforms, Enterobacteriaceae, *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens*, and spoilage bacteria (Abotchi, 2010; Bourdin 2010; Topic Popovic *et al*. 2010; Borges *et al*., 2014; Dib, 2014; Kpodékon *et al*., 2014; Gauthier, 2015). Thus, the different resulting alterations can influence the texture of fish flesh and change the taste at consumption. It appears therefore necessary to carry out a study in order to guarantee not only the health safety, but also the nutritional and organoleptic values. The objective of the study is to evaluate the effect of processing and preservation processes on microbiological quality of *Coptodon guineensis* and *Sarotherodon melanotheron* in south Benin.

Material and methods

Study area

Data collection on the effect of processing and preservation processes on the microbiological quality of *C. guineensis* and *S. melanotheron* was performed from June to December 2014 at the embankment and in the kpota market in Abomey Calavi Township. This Township is located at 12 meters of altitude between 6°26' North latitude and 2°21' East longitude. It is in the Atlantic Department, South of Benin and limited to the North by the Township of Zè, to the South by the Atlantic Ocean, to the East by the Township of Sô-Ava and Cotonou, and to the West by the Township of Tori-Bossito and Ouidah. The climate is of the subequatorial type characterized by two rainy seasons and two dry seasons. The Township has two bodies of water, Nokoué Lake and Cotonou Lagoon, a sea front juxtaposed to Cotonou Lagoon, marshes, streams and swamps. These bodies of water offer the Township a very lively artisanal fishing activity. The township has also several local markets (kpota, Glodjigbé, Akassato, Zinvié and Zè). The Kpota market has a bank where caught fish in the Nokoué Lake are regularly sold. In this market fish are marketed whole or processed. Fish processing is of artisanal type. Once collected, data and samples were analyzed at the Laboratory of Animal Biotechnology and Meat Technology of the Department of Animal Production and Health of the Polytechnic School of Abomey-Calavi of the University of Abomey-Calavi.

Microbiological analyzes

A total of one hundred and twenty (120) fish samples were randomly collected as follows: 20 *S. melanotheron* and 20 *C. guineensis* from the embankment, 20 *S. melanotheron* and 20 *C. guineensis* from the market, 10 fried *S. melanotheron* and 10 fried *C. guineensis* from the market and finally 10 smoked *S. melanotheron* and 10 smoked *C. guineensis* from the market. Smoked or fried fish were not collected at the embankment.

The sampled fish were bought whole, pre-processed and processed (fried and smoked). Whole fish were fresh fish that didn't undergo any pre-processing (scaling and evisceration). Pre-processed fish were collected after scaling and evisceration by fish wholesaler women. The scaling was done using a knife and the scales were removed all along the fish body. The evisceration was done with scissors by an abdominal incision performed in front of the anus.

Then the internal organs (digestive tract, liver, spleen, heart) were removed from the abdominal cavity. As for the fried and smoked fish, they were collected from the processors after production in open air, without any protection in the kpota market.

The sampled fish were transported to the laboratory (LBATV) in sterile Stomacher bags put in cooler containing ice at 4°C as prescribed by ISO 7218: 2007. The superficial and deep parts including skin and flesh of whole, pre-processed, smoked and fried fish were aseptically collected with sterile knives and pincers near the flame of a bunsen burner for microbiological germs investigation. For each fish, 25 g of skin and flesh were collected using a sterile knife at the first centimeter of surface at five (5) different randomly selected locations. Then, the sampled fraction was used for the stock solution preparation (ISO 7218: 2007 Standards).

From the stock solution, decimal dilutions were performed. One milliliter of the stock solution is taken and put into a test tube containing 9 ml of buffered Sodium Chloride Peptone Solution (BPW OXOID CM0509) to obtain the 10^{-1} solution. From this tube, 1ml is taken and introduced into another test tube containing 9 ml of TS in order to have the 10^{-2} solution.

The same operation is made to get the solution 10^{-3}. The isolation and enumeration of the Total Aerobic Mesophilic Flora (TAMF) was performed in accordance with ISO 4833 (2003). Fecal coliforms were counted in accordance with ISO 4831 (2006) and suspected pathogens staphylococci (*Staphylococcus aureus*) according to ISO 6888-1 (1999). Sulfite-Reducing Anaerobes (SRA) and Salmonella were counted respectively according to ISO 15213 (2003) and ISO 6579 (2007). Finally, total coliforms (TC), fecal coliforms (CF) and *Escherichia Coli* were counted according to ISO 4831 (2006), ISO 7251 (2005) and ISO 7251 (2005), respectively.

Interpretation method of microbiological results

The microbiological results were interpreted according to a 3 classes plan for the Total Mesophilic Aerobic Flora, Fecal Coliforms and Staphylococci, taking into account the criteria of the Regulation N° 2073/2005 of the European Union and of the Commerce and Distribution Federation Companies (CDFC, 2014, 2016).

The samples were appreciated as satisfactory (S), acceptable (A) or unsatisfactory (NS). The criteria for each category of appreciation are given in Table 1. Concerning Salmonella and Sulfite-Reducing Anaerobic germs, the interpretation was done according to a 2 classes plan (presence or absence). Thus, the presence of salmonella or SRA in the fish flesh indicates that its quality is not satisfactory and if not, it is satisfactory. In the absence of standards used for fried fish, those of smoked fish were adapted to fried fish in the study.

Table 1. Interpretation criteria of the microbiological controls results.

Type of fish	Interpretation plan	TMAF (CFU/g)	Fecal coliform (CFU/g)	Salmonella/ 25g	SRA (CFU/g)	*Staphylococcus aureus* (CFU/g)
Fresh or frozen fish	m	10^6	10	Absence	30	10^2
	M	10^7	100			10^3
Smoked fish	m	10^6	Absence	Absence	Absence	10^2
	M	10^7				10^3

TMAF: Total Mesophilic Aerobic Flora; SRA: Sulfite- Reducing Anaerobes; CFU/g: Colony Forming Unit per gram; m = threshold below which all results are considered satisfactory; M = acceptability threshold, beyond which the results are no longer considered satisfactory

Sources: Regulation N° 2073/2005; CDFC (2014, 2016).

Statistical analysis

A variance analysis was used to test the significance of species and treatment mode effects on the hygienic traits of the two fish species flesh. The means were calculated and compared pairwise using the student t-test. The frequencies of SRA and counted salmonella were calculated by the *Proc freq* procedure of SAS (2013) and compared using the Chi-square test and the bilateral z-test.

Results

Effect of processing mode on bacterial load

The results of the effect of processing mode on the bacterial load of the two fish species are presented in Table 2. Processing mode had no effect on the TMAF, fecal coliforms and SRA loads (P>0.05). Total coliform and *Staphylococcus aureus* loads varied according to the processing mode (P<0.05). Concerning total coliforms, fried fish load ($0.017x10^3$ CFU/g) was lower (P<0.01) than those of whole ($1.23\ x10^3$ CFU/g), smoked ($0.91x10^3$ CFU/g) and pre-processed ($1.32\ x\ 10^3$ CFU/g) fish. As for *Staphylococcus aureus,* the whole fish load ($0.47x10^3$ CFU/g) was lower than that of smoked fish ($1.29x10^3$ CFU/g). Staphylococcus and SRA were not observed in fried fish.

Effect of species, locality and sex on fish bacterial load

The table 3 presents the bacterial loads by species, locality and sex of fish. The TMAF, total coliforms, staphylococcal and SRA loads were not different (P>0.05) according to the species (*C. guineensis* and *S. melanotheron*), locality (embankment and market) and sex (male and female). On the other hand, the fecal coliforms load of *S. melanotheron* was significantly higher (P<0.05) than that of *C. guineensis* ($0.738x10^3$ *vs* $0.321x10^3$ CFU/g). The TMAF, total coliforms, fecal coliforms and *Staphylococcus aureus* loads of *S. melanotheron* were higher than those of *C. guineensis* although these differences were not significant.

Effect of interaction between processing mode and species on the bacterial loads

The interaction between processing mode and fish species was significant for fecal coliforms (P<0.05). In whole fish, *S. melanotheron* had a higher fecal coliforms load (P<0.05) than *C. guineensis* ($1.12x10^3$ *vs*. $0.545x10^3$ CFU/g), and in smoked fish, the fecal coliforms load of *S. melanotheron* ($0.788x10^3$ CFU/g) was also higher (P<0.05) than that of *C. guineensis* ($0.028x10^3$ CFU/g) (Table 4). However, the fecal coliforms load of *S. melanotheron* was not different from that of *C. guineensis* in pre-processed and fried fish (Table 4).

Effect of species and processing mode on the Salmonella frequency

The percentage of identified salmonella in *S. melanotheron* (26.67%) was identical (P>0.05) to that recorded in *C. guineensis* (30%). The highest salmonella percentage was obtained in pre-processed samples (50%) and the lowest (P<0.05) were recorded in fried (10%) and smoked (10%) fish. The salmonella levels of whole fish and processed fish (smoked and fried) were similar (Table 5).

Fish microbiological quality

The table 6 presents fish microbiological quality and contamination level. The microbiological analyzes showed that 100% of the analyzed fish (whole, fried, smoked and pre-processed) obtained a satisfactory microbiological quality considering spoilage and pathogens germs (TMAF and *Staphylococcus aureus*). On the contrary, for fecal contamination germs (fecal coliforms), 55% of whole fish and 60% of pre-processed fish were of poor microbiological quality (unsatisfactory). Whole (25%), fried (10%), smoked (10%) and pre-processed (50%) fish were judged of unsatisfactory microbiological quality due to the salmonella presence. Futhermore, all the whole, fried and pre-processed fish were of satisfactory microbiological quality for SRA except the smoked fish of which 20% were of unsatisfactory microbiological quality.

Table 2. Effect of processing mode on the bacterial load.

Treatment	Total Mesophilic Aerobic Flora (10^3 CFU/g)		Total coliforms (10^3 CFU/g)		Fecal coliforms (10^3 CFU/g)		*Staphylococcus aureus* (CFU/g)		Sulfite-Reducing Anaerobes CFU/g	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Whole fish	161.87a	32.71	1.23a	0.135	0.831a	0.134	0.47b	0.31	0.23a	0.15
Fried fish	43.69a	79.52	0.0171b	0.330	0.121a	0.325	0.00c	0.00	0.00a	0.00
Smoked fish	151.94a	48.92	0.918a	0.203	0.346a	0.200	0.06c	0.46	0.22a	0.22
Pre-processed fish	211.80a	30.55	1.322a	0126	0.822a	0.125	1.29a	0.29	0.27a	0.14
ANOVA	NS		**		NS		*		NS	

NS: P>0.05; *: P<0.05; **: P<0.01; SE: Standard Error; Means between the classes of the same column followed by different letters differ significantly at the threshold of 5%; CFU/g: Colony Forming Unit per gram; ANOVA : Analysis of Variance

Table 3. Effect of species, location and sex on the bacterial load.

Variation source		Total Mesophilic Aerobic Flora (10^3 CFU/g)		Total coliforms (10^3 CFU/g)		Fecal coliforms (10^3 CFU/g)		*Staphylococcus aureus* (CFU/g)		Sulfite-Reducing Anaerobes CFU/g	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Species	*Sarotherodon melanotheron*	150.26a	35.25	0.869a	0.146	0.738a	0.144	0.62a	0.33	0.11a	0.16
	Coptodon guineensis	134.39a	32.78	0.875a	0.136	0.321b	0.134	0.15a	0.31	0.20a	0.15
Locality	Berge	110.41a	40.23	0.878a	0.166	0.628a	0.164	0.34a	0.38	0.02a	0.18
	Marché	174.24a	26.67	0.866a	0.110	0.432a	0.109	0.43a	0.25	0.30a	0.12
Sex	Femelle	111.85a	43.23	0.717a	0.179	0.462a	0.177	0.20a	0.40	0.33a	0.19
	Mâle	172.80a	27.50	1.027a	0.114	0.598a	0.112	0.57a	0.26	0.06a	0.24
ANOVA		NS		NS		*		NS		NS	

NS: P>0.05; *: P<0.05; SE: Standard Error; Means between the classes of the same column followed by different letters differ significantly at the threshold of 5%; CFU/g: Colony Forming Unit per gram; ANOVA: Analysis of Variance.

Table 4. Effect of interaction between processing mode and species on the bacterial loads.

Variation source		Total Mesophilic Aerobic Flora (10^3 CFU/g)		Total coliforms (10^3 CFU/g)		Fecal coliforms (10^3 CFU/g)		*Staphylococcus aureus* (CFU/g)		Sulfite-Reducing Anaerobes CFU/g	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Whole fish	*S. melanotheron*	174.67a	50.14	1.20a	0.208	1.12a	0.20	0.93a	0.47	0.05a	0.22
	C. guineensis	149.067a	42.03	1.26a	0.174	0.54b	0.17	0.00a	0.39	0.40a	0.19
Fried fish	*S. melanotheron*	64.013a	109.11	0.028a	0.036	0.24a	0.44	0.00a	0.00	0.00a	0.00
	C. guineensis	23.38a	80.40	0.24a	0.333	0.0047a	0.32	0.00a	0.00	0.00a	0.00
Smoked fish	*S. melanotheron*	137.61a	67.57	1.20a	0.280	0.79a	0.28	0.07a	0.63	0.10a	0.30
	C. guineensis	166.28a	73.63	0.63a	0.305	0.028b	0.01	0.06a	0.69	0.34a	0.33
Pre-processed fish	*S. melanotheron*	224.74a	44.79	1.27a	0.185	0.81a	0.18	1.90a	0.42	0.42a	0.20
	C. guineensis	198.87a	198.86	1.36a	0.177	0.83a	0.17	0.67a	0.40	0.12a	0.19
ANOVA		NS		NS		*		NS		NS	

NS: P>0.05; *: P<0.05; SE: Standard Error; Means between the classes of the same column followed by different letters differ significantly at the threshold of 5%; CFU/g: Colony Forming Unit per gram; ANOVA: Analysis of Variance.

Table 5. Salmonella frequency by species and processing mode.

Variation source		Number	Salmonella spp (%) Présence	CI
Species	*S. melanotheron*	60	26.67a	15.83
	C. guineensis	60	30a	16.40
Treatments	Whole fish	40	25ab	18.98
	Fried fish	20	10a	18.59
	Smoked fish	20	10a	18.59
	Pre-processed fish	40	50b	21.91

Percentages between the classes of the same column followed by different letters differ significantly at the threshold of 5%; CI: Confiance Interval; %: percentage.

Table 6. Fish contamination level and fish microbiological quality.

Variables		Mean (10^3 CFU/g)	Appréciations critera CFU/g	Number	Interpretation A (%)	S (%)	NS(%)
Total Mesophilic Aerobic Flora (TMAF)	Whole fish	161.87	F≤ 3.10^6 ; 3.10^6 <F≤ 10^8 ; F>10^8	40	0	100	0
	Fried fish	43.69		20	0	100	0
	Smoked fish	151.94		20	0	100	0
	Pre-processed fish	211.8		40	0	100	0
Fecal coliforms	Whole fish	831.30	F≤ 30 ; 30 <F≤ 10^3 ; F>10^3	40	35	13	55
	Fried fish	121.24	Absence	20	10	90	0
	Smoked fish	346.15	Absence	20	0	100	0
	Pre-processed fish	822.30	F≤ 30 ; 30 <F≤ 10^3 ; F>10^3	40	30	10	60
Staphylococcus aureus	Whole fish	0.47	F ≤3.10^2	40	0	100	0
	Fried fish	0.00		20	0	100	0
	Smoked fish	0.06		20	0	100	0
	Pre-processed fish	1.29		40	0	100	0
Sulfite-Reducing Anaerobes (SRA)	Whole fish	0.23	F ≤ 90	40	0	100	0
	Fried fish	0.00	Absence	20	0	100	0
	Smoked fish	0.22	Absence	20	0	80	20
	Pre-processed fish	0.27	F ≤ 90	40	0	100	0
Salmonella spp	Whole fish	25	Absence/25g	40	0	0	100
	Fried fish	10		20	0	0	100
	Smoked fish	10		20	0	0	100
	Pre-processed fish	50		40	0	0	100

S: Satisfactory; A: Acceptable; NS: Not satisfactory; CFU/g: colony forming unit per gram; F: Flora, microbiological load; m: threshold below which results are considered satisfactory; M: threshold of acceptability beyond which results are no longer considered satisfactory.

Discussion

Total coliforms and TMAF loads of whole, pre-processed, and smoked fish were higher than that of fried fish. The obtained loads are lower than those defined satisfactory by the Regulation 2073/2005/EC and are lower than those obtained by Barro *et al* (2007) who worked on freshwater fish (catfish and carp) in Burkina. They recorded in fresh fish TMAF (8.1×10^8 CFU/g) and total coliforms (6.8×10^5 CFU/g) loads higher than those in fried fish (2.7×10^7 CFU/g and 7.3×10^3 CFU/g respectively). The observed differences may be related to the effect of fish pre-processing (evisceration, scaling), processing mode (frying and smoking) and processing conditions.

Improper evisceration and scaling expose more the fish flesh to microbial contamination and high load, because of evacuated viscera not free of germs and flesh injuries caused by the scaling. When these fish are smoked, the initial germ loads decrease as a result of the compounds generated by the combustion combined with the temperature. Similarly, frying destroys germs through the frying temperature. In this study, the frying temperature would be higher than that of the smoking. Generally, temperature during hot smoking varies between 40 and 100°C (Arvanitoyannis and Kotsanopoulos, 2012) and that of frying between 160 and 195°C (FAO/OMS, 2009). This explains the lower germ loads obtained in the current study in fried fish compared with smoked fish.

In addition, contamination by unhygienic dirty materials may vary from one processing place to another and may explain the high germ load after fish smoking.

It was observed that pre-processed fish (eviscerated, scaled) had a higher microorganism load than the whole fish. This increasing load may be due to the fish contamination by viscera germs. Cases of flesh fish contamination by intestinal microflora during evisceration have been reported by Lefèvre and Bugeon (2008), Sujatha *et al.* (2011) and Sichewo *et al.* (2014). Austin (2006) and Dauchy Silbande (2016) reported that these microorganisms are found on the entire outer surface (skin and gills) and in intestines of living and freshly caught fish. Their loads vary enormously from 10^2 to 10^7 CFU/cm^2 on the surface of the skin and from 10^3 to 10^9 CFU/g in the gills and intestines (Adam and Moss, 2008). The obtained charges in this study are between the microorganisms' proportions recorded by Adam and Moss (2008). In addition, the contamination during evisceration was evoked by Viji *et al.* (2014) in a study carried out in India on gutted and ungutted *Pangasianodon hypophthalmus* (catfish). In their study, the eviscerated *P. hypophthalmus* showed a high bacterial load compared to the non-eviscerated ones. Pre-processing (evisceration and scaling) is a route of fish contamination.

Concerning the processing mode effect, lower microorganisms' loads in fried and smoked fish compared to pre-processed and whole fish were observed. Same trends of germs reduction were found during the fish smoking (Djinou, 2001; Goueu, 2006; Abochi, 2010; Degnon *et al*., 2013 and Chabi *et al*., 2014). This load reduction is due on the one hand to the temperature (heat) effect which considerably reduces the microbial load during frying and on the other hand to the smoke antibacterial effect added to the heat in the case of smoked fish. According to Abochi (2010) and Zuraida *et al*. (2011) hot smoking destroys microorganisms and smoke can play an antiseptic role due to the phenolic fraction of low boiling level. ANSES (2010) reinforces this idea and stated that heat differently affects microorganisms depending on the species, the strain, even their number and vegetative forms resist less than sporulated forms. This observation explains the differences in obtained loads from one microorganism to another. Moreover, Knockaert *et al*. (2009) explained that smoking slightly acidifies the flesh, which slows down microbial growth. In the current study, acidification may also explains the low obtained loads in smoked fish in contrary to those obtained in whole and pre-processed fish.

Whatever the processing mode, contaminations with fecal coliforms and Salmonella were found in this study Similar results indicating fecal coliforms presence in samples were reported by Wabi (2010), Degnon *et al*. (2013) and Chabi *et al*. (2014). On the contrary, no Salmonella was found by Kpodékon *et al*. (2014) in smoked mackerel (*Trachurus Trachurus*) in the markets of Abomey Calavi, Cocotomey and Godomey in Benin, by Dodd (1992) in smoked fish in Toronto, by Oulaï *et al.* (2007) in *Ethmalosa fimbriata* and *Sardina aurita* traditionally smoked in Côte d'Ivoire, by Abotchi (2010) in artisanal smoked fish in Togo. Djinou (2001) found a frequency of 0.8% compared to the 10% obtained for frying and smoking in the present study. Because of the presence of *Salmonella spp*. and the proportions of fecal coliforms found in whole, pre-processed and smoked fish samples, they are declared of unsatisfactory microbiological quality and unsuitable for consumption in accordance with Regulation 2073/2005/EC which doesn't allow the presence of fecal germs and Salmonella. The fecal germs presence is a health risk for the consumer. Fecal coliforms and Salmonella contamination in this study is certainly related to poor hygiene during treatment (scaling and evisceration) and processing (smoking and frying) and poor microbiological quality of the

water used for fish treatment, which is polluted by garbage, excrement, urine of residents and chemical pollutants (Kinsiclounon *et al*., 2013; Adjagodo *et al*., 2016; Goussanou *et al*., 2018). Also hands and surfaces contamination during washing and evisceration of fish is a common route of infection for these fish.

Conclusion

The study carried out on the effect of processing and preservation processes on the microbiological quality of *C. guineensis* and *S. melanotheron* in south Benin reveals that processing and preservation processes (frying and smoking) reduce spoilage and pathogens germs. Fish frying is the best method that has obtained the lowest or no load for some germs (*Staphylococcus aureus* and SRA). Production and marketing conditions do not respect good hygiene practices rules. Operations such as evisceration and scaling are also routes for fish microbial contamination. The processing processes used are faulty and constitute a risk for the consumer. The rigorous application of hygiene rules throughout the process of pre-processing, smoking, frying and fish sale could reduce the microbial flora of contamination.

Conflicts of Interest: None.

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