

Research Article

Level of Contamination and Alteration of Cuttlefish Fished and Processed in Companies in Côte d'Ivoire

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Abstract

This study was carried out from 2019 to 2021. Its aim was to assess the quality of cuttlefish intended for local and international markets in a company processing fishery products in Côte d'Ivoire. The biochemical analyses (Total Volatile Basic Nitrogen (TVBN)) were based on the distillation method of an extract deproteinised by perchloric acid (HClO_4). Microbiological analyses (total aerobic mesophilic flora (TAMF), staphylococci (SPP), anaerobic sulphite-reducing bacteria (RAS), faecal coliforms (FC) and salmonella) involved isolation and identification techniques. The various analyses carried out by the National Laboratory for Support to Agricultural Development (LANADA) made it possible to assess the median contamination values and satisfaction percentages (TAMF, FC, SPP), which are respectively $2.2 \cdot 10^4$ CFU/g with 94%; less than 10 CFU/g with 94%; less than 100 CFU/g with 100%. RSA and salmonella were absent in all the products analysed. The temperature of the cuttlefish was high just after processing. The maximum temperature value (26.4°C) was obtained after processing, while the minimum temperature value (1.6°C) was recorded during storage in a positive cold room. However, it is essential to improve manufacturing practices and preservation methods in order to avoid poisoning the population. These data should also encourage fishermen to practise good preservation methods.

Keywords

Microbiological Contamination, Biochemical Spoilage, Processed Cuttlefish, Côte d'Ivoire

1. Introduction

Fisheries resources play an important role in people's lives. They represent not only food security, but also economic and social security [1]. But the unregulated exploitation of these resources leads to a drop in production. The increasing exploitation of fishery resources and the depletion of a number of large fish stocks that once supported industrial fishing, are forcing closer attention to unsuitable marine resources, which include many species of cephalopods such as additional or alternative source of protein to fish [2]. Cuttlefish species are fished in many parts of the world [3]. The main producers in

2018 were by far China (33%) and India (22%). Over the past decade (2009-2018), reported catches of cuttlefish species have increased by 25%. According to EUROSTAT, landings of cuttlefish species in the EU amounted to 23,170 tons for a total value of €158 million. However, imports of cuttlefish (mainly frozen) exceeded EU production, with 37,968 tons [3]. The Ministry of Animal Production and Fisheries Resources says that overall marine fisheries resources in Côte d'Ivoire are very limited and threatened by unreported and unregulated illegal fishing practices [4].

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In the exclusive economic zone of Côte d'Ivoire, cephalopods constitute an important halieutic potential after fish. On average 1,047 tons of cuttlefish were landed daily at the industrial fishing port of Abidjan during the year 2015 [5].

Currently, many studies focus on the state of cephalopod stocks with a view to implementing rational management and assessing the risks of overexploitation [6-8].

Cuttlefish today occupies an important place in various world and national markets. This position has led to increased research in recent years. Much work has been devoted to the study of the biology of the common cuttlefish (*Sepia officinalis*) in several Mediterranean and Atlantic areas focusing on different aspects: reproductive biology [9-11], feeding behavior [12-14], population dynamics [15].

But the study on the biochemical and microbiological quality carried out by several authors is still relevant. It is essential and continues for us to carry out this study on the level of contamination of cuttlefish, in the sense that it is linked to the health of

populations. The various analyzes carried out by the National Laboratory for Support to Agricultural Development (LANADA) with within it the Central Laboratory for Food Hygiene and Agro-Industry (LCHAI), made it possible to locate the levels of contamination. Specifically, this study will assess the level of bacterial contamination and the content of Total Volatile Basic Nitrogen (TVBIN) of cuttlefish received by a company in Côte d'Ivoire.

2. Materials and Methods

2.1. Study Area

This study took place in Côte d'Ivoire, in the city of Abidjan precisely in the commune of Treichville with coordinates (5° 18' 20. 887"N and 3° 59' 58. 193"W) (Figure 1).

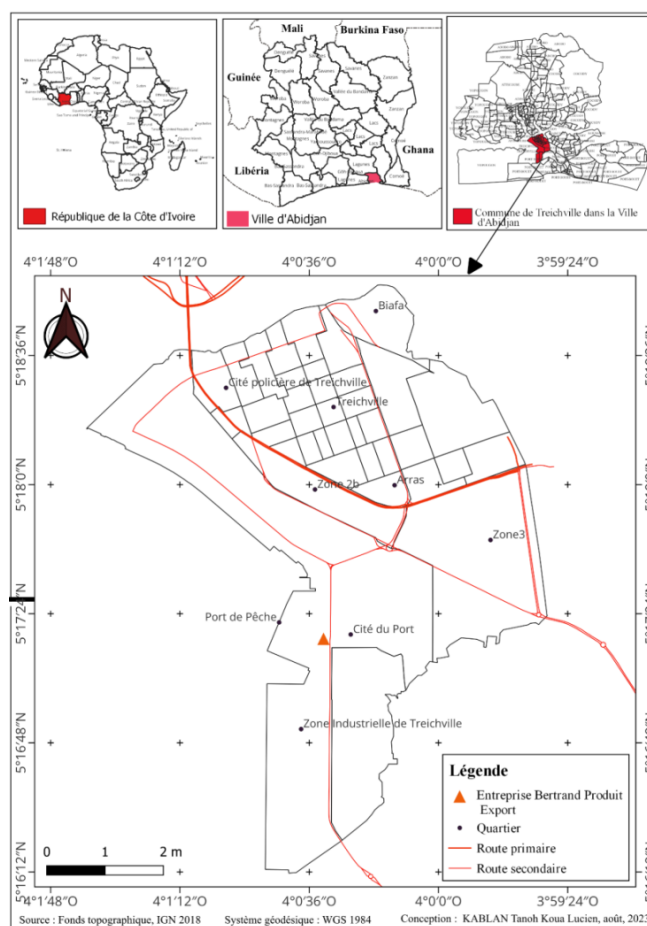


Figure 1. Map to locate the company (▲) in Treichville (KABLAN, 2023).

It was carried out on the one hand, largely in the company and on the other hand, at LANADA in the Central Laboratory for Food Hygiene and Agro-Industry (LCHAI) for carrying out the analyses. This company is a private establishment in charge of exporting fishery products (fish, crustaceans and

cephalopods). It is located in the port area of Abidjan (Côte d'Ivoire). This company is an establishment for the processing and sale of seafood products on the European Union market and on the national market.

2.2. General Information on Cuttlefish

The body consists of two parts: the head and the mantle. The head has a crown of 8 arms surrounding the mouth. It also carries two retractile tentacles in the pouches, furnished with suckers only at their extremity (tentacular club) [16]. The cuttlefish laterally has two large eyes with W-shaped pupils covered with a transparent membrane. The striped mantle covers the calcareous bone or sepien, which constitutes the internal shell of this mollusc [16]. The example of the species *Sepia officinalis* is presented by (Figure 2).



Figure 2. *Sepia officinalis* (Linnaeus, 1758).

2.3. Materials

The material consists of samples of frozen cuttlefish (Figures 3 and 4) taken from the warehouse of a company in Côte d'Ivoire. Samples are collected and packaged in sterile pouches under aseptic conditions. They are then weighed with a GRAM S5i electronic scale and packaged in a portable polystyrene cooler with the addition of frozen ice cream. Each batch sampled consists of 5 to 10 individuals of cuttlefish sampled from 2019 to 2021, between 500 g and 800 g each on a tonne of goods for self-checking analyses.

2.4. Methods

A total of 575 microbiological analyzes and 80 biochemical analyzes were carried out. The microbiological analyzes (115 samples) focused on 5 germs including Total Mesophilic Aerobic Flora, faecal coliforms, presumed pathogenic Staphylococci, sulphite-reducing anaerobic bacteria and salmonella.

A stock solution consisting of ten (10) grams of cuttlefish meat and 90 ml of a buffered peptone water (EPT) solution are taken and then followed by decimal dilutions according to the NF ISO 6887-3 standard of January 2004 where one milliliter of the stock solution is taken and introduced into a test tube containing 9 ml of tryptone salt (TS). Microbiological analyzes make use of isolation and identification techniques and counting techniques. The biochemical analyzes focused on the determination of total Volatile Basic Nitrogen (TVB-N). For Total Volatile Basic Nitrogen, the method of

distillation of a deproteinized extract with perchloric acid (HClO_4) then steam distillation, followed by its neutralization with hydrochloric acid (HCl) was used.



Figure 3. Frozen Cuttlefish Blanks.



Figure 4. Frozen raw cuttlefish.

2.5. Microbiological and Biochemical Criteria

The microbiological and biochemical criteria used are those established by Regulation (EC) No. 1441/2007 of December 5, 2007 amending Regulation (EC) No. 2073/2005 concerning microbiological criteria applicable to foodstuffs [17]. As well as those established by the Central Laboratory for Food Hygiene and Agro-Industry (LCHAI) within the National Laboratory for Agricultural Development Support (LANADA).

Table 1 and Table 2 respectively show the microbiological (m) and biochemical criteria used to interpret the results.

Table 1. Microbiological criteria (m) sought per gram of bacteria (EC, 2007).

**TAMF (CFU/g)	**FC (CFU/g)	SPP (CFU/g)	**ASR (CFU/g)	App	salmonella (CFU/25g)
$F \leq 5.10^5$	$F \leq 20$	$F \leq 10^2$	$F \leq 2$	Satisf	Absence in 25g
$5.10^5 < F \leq 5.10^6$	$20 < F \leq 2.10^2$	$10^2 < F \leq 10^3$	$2 < F \leq 20$	Acc	
$F > 5.10^6$	$F > 2.10^2$	$F > 10^3$	$F > 20$	Not satisf	

** LCHAI criterion; Acc: Acceptable; App: Appreciations; ASR: Sulfite-reducing anaerobes; CFU: Colony Forming Units; CF: faecal coliforms; F: Flora; TAMF: Total Aerobic Mesophilic Flora; Not satisf: Not satisfying; Satisf: Satisfying; SPP: Presumed Pathogenic Staphylococci.

Table 2. Criteria for TVB-N content at LANADA.

organization	Cuttlefish	Appreciation
Biochemical qualities	** TVB-N	
	$\leq 35\text{mg}/100\text{g}$ of flesh	Satisfying
Reference threshold	$> 35\text{mg}/100\text{g}$ of flesh	Not satisfying

TVB-N: Total Volatile Basic Nitrogen

3. Results

3.1. Temperatures Recorded on Cuttlefish

During the period of this study, we monitored the temperature of the products on receipt, during storage in a positive cold room and after processing. The median temperature of cuttlefish at reception, during storage in a positive cold room and during processing varied (Table 3).

Table 3. Temperatures (T°) of cuttlefish at reception, storage in positive chamber and processing in 2019, Max= maximum; Min = minimum and Med = median: median values with a letter (a or b or c) differ significantly (Kruskal-Wallis test, $p < 0.05$).

Products	Conditions	Levels	Temperature ($^\circ\text{C}$)
Cuttlefish	Reception	Max	14.9
		Min	11.6
		Med	13.5 a
	Preservation	Max	3.2
		Min	1.6
		Med	2.3 b

Products	Conditions	Levels	Temperature ($^\circ\text{C}$)
	Processing	Max	26.4
		Min	20.3
		Med	22.95 c

The maximum temperature value (26.4°C) was obtained after processing, while the minimum temperature value (1.6°C) was recorded during storage in a positive cold room. The temperatures determined on receipt, during storage and after processing of the cuttlefish differed significantly from one another (Kruskal-Wallis test, $p < 0.001$).

3.2. Quality of Frozen Cuttlefish

3.2.1. Biochemical Quality

Total volatile basic nitrogen (TVBN) (Table 4) was present in all the samples analysed (frozen cuttlefish) with a median value of 13 mg of nitrogen/100 g of flesh. Of the 80 individuals analysed, 99% were satisfactory ($\text{TVB-N} \leq 35$ mg) and 1% were unsatisfactory ($\text{TVB-N} > 35$ mg). The TVB-N content had a minimum value of 5.20 mg of nitrogen/100 g of flesh and a maximum value of 82.41 mg of nitrogen/100 g of flesh.

3.2.2. Microbiological Quality

Total aerobic mesophilic flora (TAMF) (table 5), faecal coliforms (FC) (table 6), aerobic sulphite-reducing bacteria (ASR) (table 7) and presumed pathogenic staphylococci (SPP) (table 8) were present in all the samples analysed. We have respectively for cuttlefish (TAMF, CF, ASR, SPP), a median contamination value of $2.2 \cdot 10^4$ UFC/g with 94% of results satisfactory ($F \leq 5,10^5$ UFC/g) and 6% are acceptable ($5,10^5 < F \leq 5,10^6$ UFC/g); of less than 10 UFC/g with 94% of results satisfactory ($F \leq 20$ UFC/g), 3% acceptable ($20 \text{ UFC/g} < F \leq 2,10^2 \text{ UFC/g}$), and 3% unsatisfactory ($F > 2,10^2 \text{ UFC/g}$); less

than 1 CFU/g with 100% satisfactory results ($F \leq 2$ CFU/g),
less than 100 CFU/g with 100% satisfactory results ($F \leq 10^2$

CFU/g). Absence of Salmonella and RSA (contamination rate
of less than 1 CFU/g in all samples analysed).

Table 4. Alteration of cuttlefish by the level of Total Volatile Basic Nitrogen.

Alteration level	TVB-N ≤ 35 mg/100g	TVB-N > 35 mg/100g
Appreciation	satisfying	Not satisfying
	Cuttlefish	Cuttlefish
Samples	79	01
Minimum	5.20	82.41
Maximum	32.19	82.41

Table 5. Contamination of cuttlefish by Total Mesophilic Aerobic Flora.

Contamination	$F \leq 5.10^5$ CFU/g	$5.10^5 < F \leq 5.10^6$ CFU/g	$F > 5.10^6$ CFU/g
Appreciation	satisfying	Acceptable	Not satisfying
	Cuttlefish	Cuttlefish	Cuttlefish
Samples	108	07	0
Minimum	10^2	$5.2.10^5$	0
Maximum	$3.9.10^5$	3.10^6	0

Table 6. Contamination of cuttlefish by faecal coliforms.

Contamination	$F \leq 20$ CFU/g	$20 < F \leq 2.10^2$ CFU/g	$F > 2.10^2$ CFU/g
Appreciation	satisfying	Acceptable	Not satisfying
	Cuttlefish	Cuttlefish	Cuttlefish
Samples	108	04	03
Minimum	<10	10^2	$3.6.10^2$
Maximum	10	2.10^2	$1.5.10^3$

Table 7. Contamination of cuttlefish by Aerobic Sulphite-Reducers.

Contamination	$F \leq 2$ CFU/g	$2 < F \leq 20$ CFU/g	$F > 20$ CFU/g
Appreciation	satisfying	Acceptable	Not satisfying
	Cuttlefish	Cuttlefish	Cuttlefish
Samples	115	0	0

Contamination	$F \leq 2$ CFU/g	$2 < F \leq 20$ CFU/g	$F > 20$ CFU/g
Appreciation	satisfying	Acceptable	Not satisfying
	Cuttlefish	Cuttlefish	Cuttlefish
Minimum	<1	0	0
Maximum	<1	0	0

Table 8. Contamination of cuttlefish by *Staphylococci*.

Contamination	$F \leq 10^2$ CFU/g	$10 < F \leq 10^3$ CFU/g	$F > 10^3$ CFU/g
Appreciation	satisfying	Acceptable	Not satisfying
	Cuttlefish	Cuttlefish	Cuttlefish
Samples	115	0	0
Minimum	<100	0	0
Maximum	<100	0	0

4. Discussion

The median temperature of cuttlefish at reception, during storage in a positive cold room and during processing varied considerably. The maximum temperature value (26.4 °C) was obtained after processing, while the minimum temperature value (1.6 °C) was recorded during storage in a positive cold room. The temperatures determined on receipt, during storage and after processing of the cuttlefish differed significantly from one another (Kruskal-Wallis test, $p < 0.001$). There was an increase in temperature during the processing of the cuttlefish. The median temperature rose from 2.3 °C at storage to 22.95 °C during processing. But with a reduced processing time, this would avoid a rise in temperature and the multiplication of micro-organisms. Temperatures change during processing because the production time is longer.

TVB-N (Total Volatile Basic Nitrogen) is made up of all the volatile bases, including ammonia and amines. Its presence in a product is therefore directly linked to the degradation of the proteins contained in that product [18]. The median TVB-N value is 13 mg of nitrogen/100 g of meat. A comparison of our TVB-N results with the standards adopted shows that 99% of the cuttlefish samples are satisfactory. This percentage of satisfaction shows that the quality of fish products intended for export and the local market is being monitored. It reflects the proper application of hygiene rules and good manufacturing practices.

Total aerobic mesophilic flora (TAMF) is a group of germs

that provide information on the rules of good manufacturing practice, i.e. the cleanliness of handling, storage conditions, the effectiveness of treatment processes and the freshness of products [19]. TAMF was present in all the samples analysed. A comparison of our results with the standard shows that 94% of cuttlefish are satisfactory. In fact, this rate of satisfaction with the results shows that the rules of hygiene and good manufacturing practice have been properly applied. Our satisfaction percentage (94%) is higher than those of [20, 21]. These authors, who worked in Senegal on cuttlefish quality, found the same percentages, i.e. 90% of cuttlefish were satisfactory. Our results are less satisfactory than those of [22] who worked on cuttlefish in Senegal and recorded 100% satisfactory products.

Faecal coliform (FC) or thermotolerant bacteria are an indicator of faecal contamination of the fish product after capture [22]. The 94% satisfaction rate for cuttlefish is identical to that of [20] and higher than those of [21, 22]. These authors, who worked in Senegal on cuttlefish quality, obtained 94%, 90% and 72.72% respectively. The reduction in contamination indicates that staff hygiene has been mastered.

Anaerobic sulphite-reducing bacteria (ASR) are generally clostridia whose spores are present in the external environment (soil, dust and excrement, etc.) [21]. RSA were absent in all the samples analysed. A comparison of our results with the standard shows that 100% of cuttlefish are satisfactory. These results indicate good hygiene practices. This percentage is also higher than those of [20, 21] and identical to that of [22]. These authors obtained satisfaction rates of 99%, 90% and 100%

respectively.

The presence of staphylococci (SPP) indicates post-capture contamination due to poor hygiene conditions [21]. They are of human origin (skin, nostrils, mouth, etc.) and indicate poor hygiene. Staphylococci were present in all the samples analysed. A comparison of our results with the standard shows that 100% of the cuttlefish are satisfactory. This percentage is also higher than those of [20-22]. These authors respectively obtained satisfaction rates of 91%, 90% and 91% for cuttlefish.

Salmonella is a germ that is common to all animal species and can be found in polluted environments. Its presence in food indicates a lack of hygiene [23].

All the results of the salmonella test complied with current standards: no salmonella in 25g of product. The work of [20-22] gave the same results. These results demonstrate that the cold chain is respected, as well as the correct application of hygiene rules.

5. Conclusion

Frozen cuttlefish intended for the local market and for export were subjected to biochemical and microbiological analyses in a company in Côte d'Ivoire. This study made it possible to assess their level of contamination and spoilage. The various analyses carried out by LANADA show that these products are less contaminated overall. Cuttlefish are satisfactory in terms of total volatile basic nitrogen (TVBN), faecal coliforms and staphylococci at 99%, 94% and 94% respectively. Anaerobic sulphite-reducing bacteria and salmonella were absent in all the products analysed. In view of these results, overall efforts have been made by fishermen and by the company responsible for processing these fish products. These companies need to make greater efforts to improve hygiene quality in order to increase market confidence and prevent food poisoning among the general public. However, this work needs to be followed up with further research into the value of cuttlefish, and fishermen need to be made more aware of their storage conditions in order to guarantee food safety.

Abbreviations

ASR	Sulfito-Reducing Anaerobes
CFU	Colony Forming Units
FC	Faecal Coli-Forms
TAMF	Total Aerobic Mesophilic Flora
PPS	Presumed Pathogenic Staphylococ-ci
TVB-N	Total Volatile Basic Nitrogen
LCHAI	Central Laboratory for Food Hygiene and Agro-Industry
LANADA	within the National Laboratory for Agricultural Development Support

Author Contributions

Goore Bi Gnamien Willy Taunin: ideas, formulation and general objectives of the research, supervisory responsibility, general supervisor of the article

Kablan Tanoh Koua Lucien: Research and survey process, data collection and management, principal researcher who wrote the initial article, application of statistical techniques

Kouame Toto: Secondary investigator, participation in the writing and finalization of the manuscript, technical supervisor of the main author and corresponding author

Conflicts of Interest

The authors declare no conflicts of interest.

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