



Diagnostic of the Cooking and Storage Systems of a Cassava-Derived Food Locally Called “fufu” in the City of Yaoundé, Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JJBA, AMBB and RHH designed the study, wrote the protocol, collected information from participants and wrote the first draft of the manuscript. Authors MWN and HTM performed statistical analyses of data. Authors ADKT, HTM and GNM revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Fufu is among the most consumed traditional dishes derived from cassava in Cameroon. However, the processing of cassava into flour used in the preparation of fufu, the home storage of that flour and the cooking practices might lead to microbial contamination and hence constitute a potential health risk for consumers. The aim of this study was to diagnose the cooking systems of fufu in the city of Yaoundé taking into consideration the processing of granules, its storage systems and the cooking practices. A cross-sectional study was conducted in the city of Yaoundé. A total of 74 participants were submitted to a survey by the means of a structured questionnaire. Information on the socio-demographic characteristics of the population, the nature and quality of raw material used to cook fufu, the cooking processes, the storage of cooked fufu and complaints associated with the consumption of fufu were collected and statistically analyzed. Results showed that the majority of

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participants were women (82.4%) with university education level (73%). Most of the participants preferred granules of fermented cassava as raw material. The odor was the main purchasing criteria of raw materials which were generally stored at room temperature ($25\pm 2^{\circ}\text{C}$) in the household for 1 to 2 weeks. 76.05% of participants were aware of the impact of microorganisms on the color and odor of the raw material. Two cooking processes of fufu were identified, the heating duration being the main difference. The majority of participants used a heating duration of less than 15 min as it leads to slightly rigid, less elastic and not sticky fufu highly appreciated in the Centre and Sud regions of Cameroon. Leftovers fufu eaten by participants without any heating process and leftovers fufu reheated for a short duration before being consumed might be the causes of digestive troubles associated with its consumption. Prospective studies should be conducted to optimize the cooking process of fufu taking into consideration its microbiological (sporulated microflora) and sensorial quality.

Keywords: Cassava; Storage systems, traditional dish; fufu; cooking systems; health risk.

1. INTRODUCTION

Cassava is a staple food for around 500 million of people around the world. It represents the fourth crop production in the diet of the world population after rice, wheat and maize with an annual global production estimated at 227.8 million tons in 2018 [1-2]. It is a highly perishable food that is rich in cyanogenic compounds [3]. Cassava roots are generally fermented and transformed into various by-products such as cassava stick or granules, tapioca and flour in order to reduce its toxicity and minimize the risks of spoilage and post-harvest losses [4-5]. The processing of cassava is variables from one region to another and is mainly dependent on the dietary habits of the population. The variability of the processing techniques leads to the obtention on products showing varying hygienic quality [6]. Among the products derived from fermented cassava, the flour is highly used for the preparation of a traditional dish locally called fufu.

Cassava flour is obtained from the milling of fermented cassava dried granules. Indeed, the granules result from a fermentation process of cassava which is generally done traditionally and under not controlled conditions [7]. Following the fermentation, the obtained products are air-exposed to sunlight for drying. In these conditions, the dried granules or chips are subjected to toxinogenic fungi, mycotoxins, polycyclic aromatic hydrocarbons (PAHs), as well as to numerous pathogenic and spore-forming bacteria contamination [8]. Delobe et al. [9] highlighted that the contamination of cassava chips with moulds occurs during the drying step of fermented cassava. Djoulde et al. [6] revealed the presence of coliforms, *Bacillus cereus* and *Clostridium* sp. in cassava chips and gari. These

observations questioned the safety of the resulting flour and therefore the microbiological and physicochemical quality of the fufu and its suitability for human consumption. In fact, during the cooking process of fufu, the flour is homogenized in hot water for a while, depending on the desired taste and ethnicity. The cooking process of fufu is not documented as there are several types of fufu with different color and sensorial characteristics.

In a study conducted in Zambia by Alamu et al. [10] on the consumption and use of cassava products among the population, the authors found that only 20-25% of the participants took precautions during the storage and preservation of these products. It therefore appears interesting to perform a similar study in Cameroon taking into consideration not only the storage conditions of traditional cassava derived products but also the cooking practices in order to bring light on potential health risks associated with the consumption of food made from these products. This study was therefore designed to identify the different cooking process of fufu and identify the possible critical points.

2. MATERIALS AND METHODS

2.1 Study Site and Period

The study was conducted from January to May 2021, in the city of Yaoundé, Cameroon. Yaoundé is the capital of the Centre region of Cameroon. It's located between $30^{\circ}47' - 3^{\circ}56'N$ and $11^{\circ}10' - 11^{\circ}45' E$. With an altitude of 750 m and a population of approximately 5 million inhabitants distributed over an area of 285 km². It's the political capital of the country with a cosmopolitan agglomeration because it brings

together various cultures with almost all the tribes of Cameroon [11].

2.2 Study Population

This study targeted any household with people aged 18 years and above, living in the city of Yaoundé and consuming fufu. Anyone who did not meet the inclusion criteria was excluded from this study.

2.3 Study Design

A cross-sectional study design was conducted and a convenience sampling design was used to choose participants involved in the study. Information was collected from the selected participants by the means of a structured questionnaire.

2.4 Questionnaire Design and Data Collection

A structured questionnaire was administered to the participants through a direct interview. The questionnaire had 64 questions regrouped into 4 sections. The first section was focused on the demographic data of the respondents. The second one was on the quality criteria of the raw material used to cook fufu (purchasing, processing and storage) and knowledge on microorganisms as well as their impact on food. The third section presented information on the cooking practices of fufu and the management of its leftovers. The fourth section concerned the complaints associated with the consumption of fufu. The questionnaire was pre-tested with a few proportions of the population living in the city of Yaoundé in order to collect any new information which was in direct relation with the objectives of the study. A total of 74 participants were selected for the study using the convenience sampling method. They were interviewed and data recorded.

2.5 Statistical Analysis

Data collected from the survey were edited, entered in an Excel sheet and coded. They were then exported to the Statistical Package for Social Sciences (SPSS) version 25.0 software where descriptive statistics were applied to summarize data as frequencies and percentages. Chi square (χ^2) test was used to assess the relationship between categorical variables. Statistical significance of

variables was set at $p < 0.05$. Graphs were plotted using Microsoft Excel 2016 software.

3. RESULTS

3.1 Socio-Demographic Characteristics of the Study Population

Among the 74 participants involved in this study, 82.4% were women and 17.6% were men (Table 1). 58.1% of the participants were between 30-49 years old, while about 30% were between 18-29 years and only 10% were aged of 50 years and more. Regarding marital status, 60.4% were single while about 39.6% were either married or cohabiting and just 5% were widowed or divorced. Over 70% of the participants included in the survey had a university education level, while the rest had only secondary education level (21.6%) or no education level (5.4%). Furthermore, those who were employed were the most represented with 60.6% and the highest class of the number of persons per household was the one with 3 to 5 people (51.4%). The majority of participants were originated from the Centre/South (40.5%) and West (32.4%) regions.

3.2 Cooking System of “fufu”

3.2.1 Nature and quality of raw material used to cook “fufu”

Table 2 shows the choice of the state of raw material used for fufu cooking as influenced by gender, marital status and the education level of participants. Globally, the raw material was available on markets in two states: as flour and as dried granules obtained from fermented cassava tubers. 62.2% of participants preferred to purchase flour while 35.1% preferred granules. As observed in Table 2, there was no significant association between the choice of the state of raw material used for fufu cooking and the sex, the marital status or the education level of participants.

However, statistical analysis revealed that there is a significant dependence between the education level of participants and the choice criteria of raw material at purchasing on markets ($\chi^2=21.44$, $p=0.006$). Odor was the most important criteria as it scored the highest prevalence.

Table 1. Socio-demographic parameters of the study population

Variables		Frequency	Percentage (%)
Sex	Male	13	17.6
	Female	61	82.4
Age	18-29 years	24	32.4
	30-49 years	43	58.1
	50 years and more	7	9.5
Marital status	Single	45	60.4
	Married/concubine	29	39.2
Education level	No formal education	4	5.4
	Secondary	16	21.6
	University	54	73.0
Profession	Non-employed	28	39.4
	Employed	43	60.6
Number of persons per household	1-2	13	17.6
	3-5	38	51.4
	6 and more	23	31.1
Region	Centre/South	30	40.5
	Far North	4	5.4
	Littoral	8	10.8
	North West/South West	8	10.8
	West	24	32.4

N=74 participants

Table 2. Association between the choice of the type of raw materials for “fufu” cooking and socio-demographic parameters of the study population

Parameters		State of raw materials		X ²	p-value
		Flour % (n)	Granule % (n)		
Sex	Male	19.6 (9)	18.1 (5)	0.196	0.658
	Female	80.4 (37)	81.9 (23)		
Marital status	Single	58.6 (27)	61.5 (18)	0.056	0.813
	Married/concubine	41.3 (19)	38.5 (10)		
Education level	No formal education	4.3 (2)	7.7 (3)	0.386	0.824
	Secondary	21.7 (10)	19.2 (6)		
	University	73.9 (34)	73.1 (19)		

N=74 participants, Values in bracket refers to the number of participants

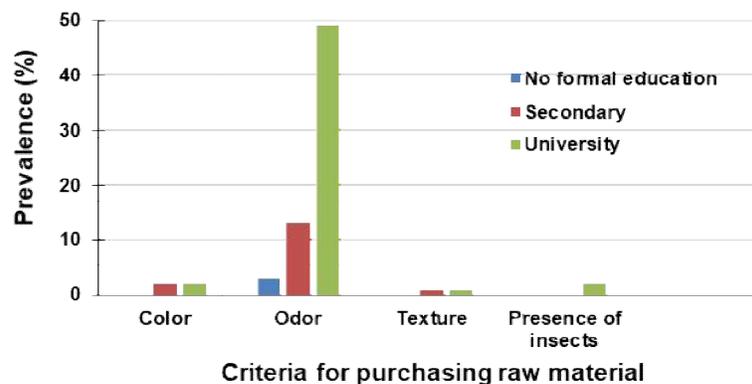


Fig. 1. Criteria used to purchase the raw material as influenced by the education level of the participants

Among the purchasing criteria, the color of raw material was found dependent on the region from which participants originated although non-significant ($\chi^2=4.543$, $p=0.805$). Two colors for the raw material were mainly recorded: white and yellow. The yellow color was mostly preferred by participants who originated from the West, North-West/South-West and Centre/South regions while the white color was preferred by those who originated from the littoral region (Fig. 2). Equal preferences to both yellow and white color were recorded with participants who originated from the Far-North region (Fig. 2).

Participants' knowledge of microorganisms is presented in Table 3. Globally, the majority of participants were aware of the existence of microorganisms independently of their education level. The percentages of participants without knowledge of microorganisms were null and void for the different purchasing criteria. This

observation demonstrates that participant's knowledge of microorganisms significantly impacted their purchasing criteria of raw material.

The potential impact of the presence of microorganisms on the quality (color and odor) of flours and/or granules of raw material used to cook "fufu" was asked to the participants during the investigation. The results of the analysis showed that 76.05% of participants declared that the presence of microorganisms can modify the color and odor of the raw material, 12.67% declared the contrary while the rest of 12.67% did not know. A repartition of this information according to the education level of participants is presented in Fig. 3. Although not significant ($\chi^2=6.085$, $p=0.193$), the majority of the participants with a university level of education knew that microorganisms could modify the color and odor of the flours or granules used as raw material to cook "fufu".

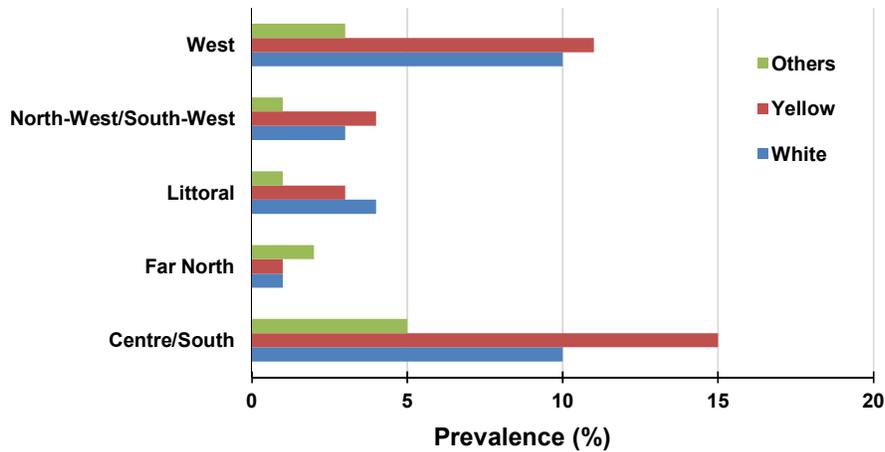


Fig. 2. Choice of the color of raw material at purchasing according to the region from which participants originated

Table 3. Relation between the purchasing criteria of raw material, the education level of the participants and their knowledge of microorganisms

Parameters		Knowledge on microorganisms		χ^2	p-value
		Yes % (n)	No % (n)		
Criteria at purchasing	Color	5.7 (5)	0 (0)	0.585	1.00
	Odor	87.1 (62)	100 (1)		
	Texture	2.9 (3)	0 (0)		
	Presence of insects	2.9 (3)	0 (0)		
Education level	No formal education	4.3(4)	0 (0)	8.799	0.066
	Secondary	21.4 (16)	100 (1)		
	University	74.3 (53)	0 (0)		

N=74 participants, Values in bracket refers to the number of participants

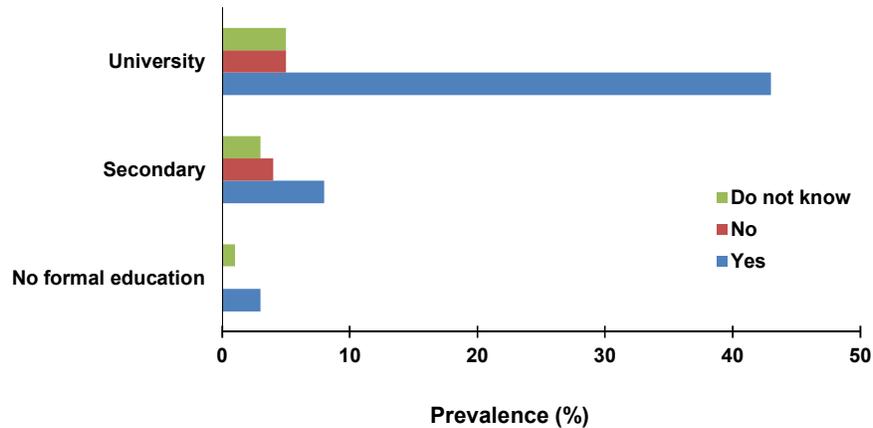


Fig. 3. Education level of participants concerning the impact of the presence of microorganisms in the raw material used to cook "fufu" on its quality

Participants declared that they stored the rest of the unused raw material at home. During their storage, some participant exposed the raw material to sunlight. Table 4 shows the storage conditions of raw material according to the education level of participants. No significant relationship between storage time, unit drying operation and level of education was noticed. However, a higher prevalence of these practices was more observed amongst people with university education level.

3.2.2 Cooking process of "fufu"

Two main cooking processes of "fufu" were identified in this study (Fig. 4). These processes included several unit operations starting from grinding or sieving depending on the state of raw material (granule or flour) to conditioning into a bowl ready to eat. For the first process used by

71.6% of participants, these unit operations were: Crushing–Sieving–Homogenization–Heating–Homogenization–Fufu. For the second one used by 28.4% of participants, it was: Crushing–Sieving–Homogenization–Heating–Homogenization–Heating–Homogenization–Fufu. The main difference between the two processes was at the step of heating. In the first process, the heating duration was up to 15 min, while in the second one, it was between 30 and 45 min.

An important unit operation during the "fufu" cooking process is the dissolution of flour in water. 8.1% of participants performed the dissolution of flour in cold water, 12.2% in lukewarm water and the great majority (79.7%) in boiling water. Even if not significant, the choice of water temperature tended to vary according to the education level and the region from which participants originated as shown in Table 5.

Table 4. Relation between the storage duration of raw material, the drying unit operation during storage and the education level of the participants

Parameters		Education level			X ²	p-value
		No formal % (n)	Secondary % (n)	University % (n)		
Storage duration	1 week	20.0 (2)	0 (0)	80.0 (8)	3.772	0.708
	>1 to 2 weeks	3.8 (1)	26.9 (7)	69.2 (18)		
	3 to 4 weeks	5.0 (1)	20.0 (4)	75.0 (15)		
	5 to 10 weeks	5.6 (1)	16.7 (3)	77.8 (14)		
Drying during storage	Yes	4.1 (1)	25.0 (6)	70.8 (17)	0.228	0.892
	No	6.5 (4)	21.7 (12)	71.7 (34)		

N=74 participants, Values in bracket refers to the number of participants

Table 5. Temperature of water during the dissolution of flour concerning the education level and the region from which participants originated

Parameters		Temperature of water			X ²	p-value
		Cold % (n)	Lukewarm % (n)	Boiling % (n)		
Region	Centre/South	16.7 (1)	44.4 (4)	42.4 (25)	6.146	0.631
	Far North	0 (0)	11.1 (1)	5.1 (3)		
	Littoral	16.7 (1)	0 (0)	11.9 (7)		
	North-West/South-West	0 (0)	11.1 (1)	11.9 (7)		
	West	66.7 (4)	33.3 (3)	28.8 (17)		
Education level	No formal education	0 (0)	0 (0)	6.8 (4)	3.164	0.531
	Secondary	0 (0)	22.2 (2)	23.7 (14)		
	University	100 (6)	77.8 (7)	69.5 (41)		

N=74 participants, Values in bracket refers to the number of participants

Succeeding the dissolution of flour in water, the mixture was homogenized. It was observed that 39.4% performed homogenization out of the fire, 40.8% on fire and 19.7% alternated homogenization out of the fire and on fire. Taking into consideration the region from which participants originated, those from Centre/South and West scored the highest percentage of homogenization out of the fire, on fire and the alternation of both methods.

Homogenization was performed using two types of pestles. The first one with a round bottom (81.70%) and the second one with a flat bottom (18.30%). The use of pestle with round bottom was significantly associated with the education level of participants ($\chi^2=10.805$, $p=0.005$) while no significant relationship between the type of pestle and the region of participants was noticed ($\chi^2=3.182$, $p=0.526$).

Heating conditions of the homogenized mixture was the main unit operation differentiating the two cooking processes of "fufu". Most of the participants heated the homogenized mixture of

flour and water at approximately 100°C duration of up to 15 min (71.6%). Only 28.4% heated at approximately 100°C for 30 - 45 min (Table 6).

Several criteria were used by the participants to check the way that after the heating process the "fufu" is ready to eat or not. Fig. 5 presents the distribution of these criteria according to the sex of participants. It emerges from this a predominance of the elasticity criterion compared to the tender, sticky and steady criterion, with a more pronounced prevalence observed in women than in men.

3.2.3 Storage of "fufu"

According to study participants, leftovers "fufu" were stored in pots, plates, trays and thermos. Before being consumed, 53.52% of participants reheated the leftovers "fufu" while 46.48% consumed leftovers "fufu" without any heating process. The majority of the participants who reheated leftovers "fufu" were having a university level of education (71.1%).

Table 6. The heating duration during "fufu" cooking with the education level and the region from which participants originated

Parameters		Heating duration		X ²	p-value
		Less than 15 min % (n)	More than 15 min % (n)		
Education level	No formal education	7.5 (4)	0 (0)	2.249	0.325
	Secondary	18.9 (10)	28.6 (6)		
	University	73.6 (39)	71.4 (15)		
Region	Centre/South	45.3 (24)	28.6 (6)	3.233	0.520
	Far North	5.7 (3)	4.8 (1)		
	Littoral	11.3 (6)	9.5 (2)		
	North-West/South-West	11.3 (6)	9.5 (2)		
	West	24.6 (14)	47.6 (10)		

N=74 participants, Values in bracket refers to the number of participants

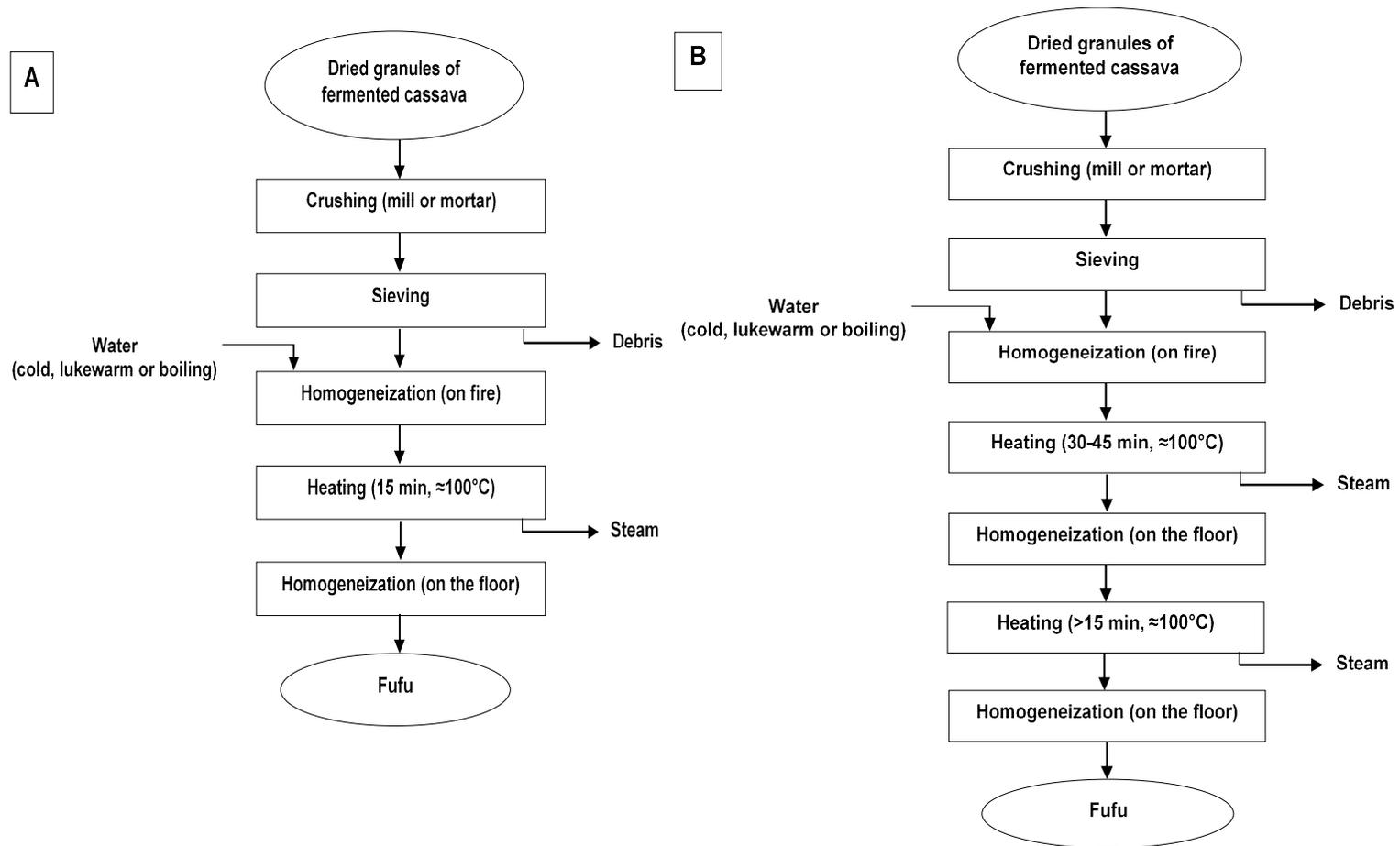


Fig. 4. Flow diagram of the first (A) and second (B) cooking processes of “fufu” identified from participants

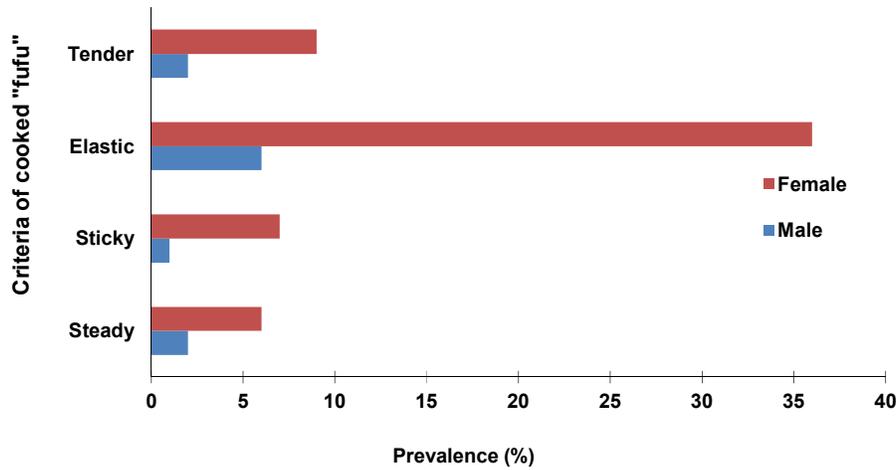


Fig. 5. Criteria of ready to eat “fufu” concerning the sex of participants

3.3 Complaints Associated with the Consumption of “fufu”

Digestive troubles following consumption of “fufu” was reported in this study mainly by participants with no formal education level (25%) while participants with secondary and university education level scored 0 and 5.8% of digestive troubles, respectively.

4. DISCUSSION

In this study, a survey was carried out on the cooking systems of Cameroonian traditional dishes locally called fufu and made from dried fermented cassava. The analysis of socio-demographic data showed that the preparation of fufu in households was mainly performed by women. This observation can be explained by the fact that according to African culture, women are those who assume the household chores in the homes and therefore the kitchen [12].

In the cooking process of fufu, the raw material used was either dried granules of fermented cassava or its flour. Both are available on markets. As observed in this study, most of the participants (63.9%) preferred purchasing the raw material in the state of flour. This can be ascribed to the lack of time and the difficulties associated with the milling of granules and the requested sieving. In fact, most of the participants were employed (60.6%) and thus do not have sufficient time to mill and sieve granules

after the work. At purchasing, some quality criteria of raw material were identified in this study with odor as the most important one. Modifications of parameters such as color, texture, presence of insects appear when the product reached a certain level of spoilage. However, odor was the most important criteria reported by participants because fermented cassava has a specific odor that is well recognized and appreciated by consumers [13-14]. Hence, a little modification of that parameter is directly detected and used as indicative of spoilage.

Regarding the preferred color of the raw material, two colors were mostly mentioned: yellow and white [15]. These colors were associated with the region of origin of the participants. Indeed, cultural affiliation has always guided consumers' choice. In the Centre region, cassava cultivation gives yellowish flesh color depending on the type of soil which most often leads to the production of equally yellowish cassava flour. In contrast, the coastal region is known for its cultivation of white melting cassava which in turn produces white flour. The consumer when going to the market will tend to take what is closest to what they know.

Unlike the participants with lower education level, those with university-level were more aware of the impact of the presence of microorganisms in the raw material on its quality attributes. This could be explained by the fact that knowledge on

microorganisms as well as their impact on the quality of the flour and the repercussions incurred by the consumers is often taught in higher schools.

Participants declared storing at home the rest of un-used raw material either in a state of granules or flour. Storage was mainly performed at room temperature. In these conditions, associated with the high level of humidity which is common in the city of Yaoundé, microorganisms such as moulds [16] can easily develop on these matrixes and produce their mycotoxins. This result is worrisome taking into consideration the thermostable character of mycotoxins and mould spores. The presence of mycotoxins in a similar kind of dish prepared with maize flour was reported by Nguégwouo et al. [17]. Hence, research should be taken on the presence of mould spores in the stored raw material used to cook fufu. Besides, during the storage, it was noticed that some participants air-exposed the raw material to sunlight. This practice can be appreciated as it reduces the humidity of the matrix and thus prevents the proliferation of microorganisms. However, despite the advantage of the air-exposition, it could lead to contamination with other microbial spores [18].

The flour resulted from the milling of granules was sieved by participants with university education level probably due to the ability of sieving to remove physical contaminants such as debris, insects and particles. This sieving practice should be applied by people who directly purchased flour on the market, as during the air-exposition selling, there are several contamination sources.

After sieving, participants dissolved flour in water at different temperatures. The dissolution of flour in cold water by some participants was surprising as it is known that starch which constitutes the majority of cassava flour is highly soluble in hot water [19,20]. The fact that the great majority performed the dissolution in hot water (79.7%) could result from their cooking practices and their education level.

As shown in the flow diagram of the fufu cooking process (Figs. 4A and 4B), the mixture of flour and water is homogenized either on fire or the floor. The role of homogenization is to completely dissolve flour in water to avoid the formation of lumps. It is important to mention that flour addition in water was gradually done during the homogenization process. It continues until the

desired texture and viscosity is reached. The choice of homogenization on or out of fire could be explained by the cultural background of each participant. Participants from Centre/South and West used homogenization out of the fire, on fire and the alternation of both methods.

Homogenization was performed using pestles for which the bottom had two different forms. According to participants, the form of pestle contributes to avoid and reduce the formation of lumps which are pouches containing flours that were not dissolved in the water. Although the use of pestle with round bottom was significantly associated with the education level of participants ($\chi^2=10.805$, $p=0.005$), it seems to avoid the formation of lumps in fufu which is the desired goal of the homogenization depends more on the technique used. This can be explained by the fact that participants using pestles with flat bottom also obtained fufu free of lumps. Hence, the focus should be on technique rather than the education level because lumps can be the reservoir of spores-forming microorganisms that are thermostable. The consumption of fufu containing lumps could lead to foodborne diseases.

The cleaning process of pestle used in the cooking process of "fufu" also represents a critical point that might lead to the continuous contamination of the cooked "fufu". Indeed, pestles are mainly made up of wood mater with several pores into which "fufu" encrusts during the preparation process and offer substrate for the growth and proliferation of microorganisms.

The homogenized mixture is heated to allow its cooking. In this study, some participants reported heating for less than 15 min (71.6%) while others heated for 30-45 min (38.4%). Among participants who heated for a duration of less than 15 min, the great majority had a university education level. This result is surprising knowing that this heating duration is not always sufficient to destroy spores-forming microorganisms that might be present in the flour. Fufu cooked in these conditions might contain microbial spores and represents a health risk for consumers. Gacheru et al. [21] had already reported the presence of spore-forming microorganisms in cassava products. Hence, it appears important to assess the spore-forming bacteria profile of fufu. An explanation of the choice of a heating duration less than 15 min could be the potential impact of heating on sensory attributes of fufu. Some participants affirmed that heating for a long

period improved the softness, texture, elasticity and stickiness of fufu which is highly appreciated in some regions of the country. In other regions such as South and Centre, heating duration is less than 15 min because participants preferred fufu which is slightly rigid, less elastic and not sticky. This observation suggests that studies must be conducted to identify optimal heating duration which satisfies both microbiological and sensorial quality of the fufu.

Leftovers of fufu stored in the household were consumed either hot (after reheating) or cold (no reheating). It is well known that handling and storage of cooked fufu could lead to its contamination if good hygiene practices were not applied. In a study conducted by Djoulde et al. [6] it was highlighted that poor handling and packaging of fufu could lead to its contamination by bacteria. This suggests that participants who have consumed leftovers fufu without any heating process are at risk of foodborne diseases. The reheating practice was mostly applied by participants with university education level (71.1%) probably because of their knowledge on the lethal effect of temperature on microorganisms. However, the impact of the reheating process of food such as rice on the proliferation of spore-forming bacteria such as *Bacillus cereus* is well known [22]. A potential risk of foodborne disease associated with the consumption of reheated fufu can therefore not be excluded. Hence, populations should be sensitized on the good reheating practices of cooked foods.

Although an outbreak of toxi-infection incriminated fufu was not officially reported, cases of digestive troubles after its consumption were declared by participants. These digestive troubles might result from contamination of flour by a spore-forming bacteria such as *Bacillus cereus* during its selling or processing. Knowing that these spores are thermo-resistant and taking into consideration the heating duration required for cooking which is less than 15 min associated with the homogenization process which might lead to the formation of lumps; it appears that these spores can be found in the fufu. The consumption of such contaminated fufu will result in digestive troubles [23]. Moreover, the leftovers fufu consumed by participants without any heating process as well as leftovers fufu reheated for a short duration before being consumed might be the causes of the digestive troubles reported in this study.

5. CONCLUSION

This study revealed two main processes for fufu cooking in households and which differentiate by the heating duration. The practices reported with the raw material (flour) and the ready to eat fufu suggest a potential health risk for consumers especially associated to microbial spores. This risk appears more pronounced with non-educated people. Prospective studies should be conducted in order to optimize the cooking process of fufu taking into consideration its microbiological (sporulated microflora) and sensorial quality.

CONSENT AND ETHICAL APPROVAL

The study was carried out according to the guidelines for human experimental models as stated by the Helsinki declaration. Besides, the ethical and administrative clearances for this study were issued by the institutional ethic committee of the Institute of Medical Research and Medicinal Plant Studies, Ministry of Scientific Research and Innovation under reference number 2017/0588/CEIRSH/ESS/MSP. Only volunteers who signed an informed consent form for their participation were enrolled.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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