



Study of the Physicochemical, Nutritional and Microbiological Quality of *Toedo* Candies Produced in Ouagadougou, Burkina Faso

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In Burkina Faso, the pulp of the baobab tree (*Adansonia digitata*) is mainly used to produce fruit juices or candies called *toedo* candies. *Toedo* candies are produced and sold in the markets and popular with the people. However, there is little data on the physicochemical, nutritional and

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microbiological characteristics of *toedo* candies produced in Ouagadougou. Thus, this study aimed to evaluate the physicochemical, nutritional and microbiological characteristics of these *toedo* candies. To carry out the study, 9 samples of *toedo* candy were taken from different producers in the city of Ouagadougou. The physicochemical and microbiological characteristics were determined by standard methods. Mineral contents were determined by flame spectrometry. The results obtained show that the *toedo* candies have an average ash content of 1.3 ± 0.21 to $1.9\pm 0.06\%$, a titratable acidity of 0.131 ± 0.001 to $0.168\pm 0.002\%$, a pH varying from 3.87 ± 0.10 to 3.91 ± 0.10 , a water content from 3.84 ± 0.00 to $16.06\pm 0.14\%$ and a dry matter (DM) content of 84.04 ± 0.14 to $96.16\pm 0.00\%$. In addition, this study revealed that these samples are rich in micronutrients such as : iron (49.30 to 127.50 mg/100 g DM), magnesium (3.53 to 42.38 mg/100 g DM), zinc (1.37 to 14.92 mg/100 g DM) and calcium (17.60 to 43.30 mg/100 g DM). Microbiological analyzes indicate the presence of total mesophilic aerobic flora (TMAF), yeasts and molds (YM) and coliforms in the samples analyzed with loads of 2.2×10^3 to 4.9×10^4 CFU/g, <10 to 2.0×10^2 CFU/g and <10 to 5.0×10^2 CFU/g respectively. For all the germs studied, 11.11% of the samples were of unsatisfactory quality, 44.44% of acceptable quality and 44.44% of satisfactory quality.

Keywords: *Toedo* candies; physicochemistry; microbiology; micronutrients; Burkina Faso.

1. INTRODUCTION

A plant of the Bombaceae family, the baobab, or *Adansonia digitata*, is a slow-growing, very massive tree that can live between 500 and 2000 years [1]. It is found in all semi-arid and subhumid regions of intertropical Africa, in Madagascar, Australia and Burkina Faso [2]. The baobab is one of the main local multi-use woody species in traditional agroforestry parks in the Sahelian countries [3]. The leaves, seeds and pulp of the baobab have been used in food and medicine for centuries in many parts of Africa [4]. The baobab is one of the most important trees for the inhabitants of three countries in the semi-arid lowland network of West Africa, namely Burkina Faso, Mali, and Niger [5]. The most used product of this tree is its fruit which contains numerous round or ovoid, hard and brownish seeds which are included in a yellowish and floury acidic pulp. The pulp of the ripe fruit appears colored, naturally dehydrated, powdery, whitish and with a slightly acidic taste [6]. Called "monkey bread", the baobab fruit is very popular in West Africa, particularly in countries such as Senegal, Mali, Guinea, Gambia, Ivory Coast and Burkina Faso. The pulp of the baobab fruit is used to make juices or dried and crushed to then be used in cooking as flour. Rich in vitamins, minerals and trace elements, this fruit is a superfood used in traditional African medicine for its many health benefits [7]. *Toedo* candy is one of the by-products made from the baobab fruit, it is a delicacy made from the baobab fruit which is widespread in the Sahel countries (Burkina-Faso, Mali, Guinea, Senegal, etc.) and is adored by the populations. In Burkina Faso, there is little data on the

physico-chemical, nutritional and microbiological characteristics of this food product. So, this study aimed to evaluate the physico-chemical, nutritional and microbiological aspects of *toedo* candies produced and sold in the city of Ouagadougou.

2. MATERIALS AND METHODS

I. Sampling: To carry out this study, 09 samples of 100 g of 03 types of *toedo* candy were collected in the city of Ouagadougou: 03 samples of plain *toedo* candy (N1, N2 and N3), 03 samples of *toedo* ginger candy (G1, G2 and G3) and 03 samples of mango and ginger *toedo* candy (MG1, MG2 and MG3). The samples of *toedo* candies from different productions were collected from food supplies and a production structure.

2.1 Physico-chemical Analyzes

2.1.1 Dry matter and water content

Dry matter was quantified according to the AOAC [8]. 5g of *toedo* candy were introduced into an empty crucible of known mass then the whole was placed in a water bath at 105°C for 4 hours. After 4 hours, the sample is left to cool in a desiccator for 15 minutes and the crucible containing the sample is weighed again. The operation was done several times in order to have a constant mass. The dry matter content was calculated according to the following formula:

$$\%MS = \frac{MF - MO}{PE} * 100$$

With :

$$\text{Ash content (\%)} = \frac{M_f - M_0}{MPE} * 100$$

MF: mass of the crucible and the sample in g
 PE: mass of the test sample in g
 MO: mass of the empty crucible
 %MS: percentage of dry matter

Mf: Final mass of the crucible sample (g)
 MO: Mass of the empty crucible (g)
 MPE: Mass of the test portion

The water content (%H) was deduced using the following formula: %H= 100-%MS

2.1.2 pH

The pH was determined according to AOAC [8] using a pH meter (HANNA instruments). Thus 5g of the sample was taken and introduced into a beaker containing 50ml of distilled water. The pH meter is first calibrated with buffer solutions (pH4 and pH7) then its electrode is introduced into the mixture after homogenization. The pH value was read on the pH meter screen.

2.1.3 Titratable acidity

The principle of titratable acidity determination is based on the neutralization of organic acids with sodium hydroxide in the presence of phenolphthalein. For this, 25 mL of distilled water was added to 2.5 g of the sample then two to three drops of Phenolphthalein were added. The mixture was homogenized using a magnetic stirrer and titrated using a 0.1 N NaOH solution. The titration was stopped at the appearance of persistent pink coloring. The TA is calculated according to the following formula:

$$\text{TA(\%)} = \frac{(V * N * 0.07)}{VP} * 100$$

V: Volume of the sodium hydroxide solution in mL poured to the equivalence; N = normality of sodium hydroxide (NaOH); VP = volume of solution (in mL) for titration; 0.07 = conversion factor in citric acid equivalent.

2.1.4 Ash content

The ash content was obtained according to the AOAC [8]. A 5g test portion (MPE) of *toedo* candy was placed in a previously weighed porcelain crucible (MO) on a balance. The crucible (with test portio) was placed in a muffle furnace at 550°C for 13 hours. At the end of the incineration, the crucible was removed and cooled in a desiccator for 30 minutes before being weighed (Mf). The ash content was calculated according to the following formula:

2.1.5 Total sugar Content

Total sugars were quantified according to the method of Montreuil and Spik [9]. Thus, 0.1g of each sample to be analyzed was finely ground in a blender then introduced into a tube. 10 mL of 60% (v/v) sulfuric acid were added to each of these tubes. The mixture was incubated in a boiling water bath (at 100°C) for 15 minutes then cooled to room temperature for 10 minutes. A dilution was made by taking 1 mL of the mixture in each tube then adding 0.5 mL of 5% phenol. After homogenization, 2 mL of sulfuric acid (H₂SO₄ 75%) was added. The tests were carried out in duplicate. The concentration of total sugars was deduced from the standard curve carried out with D-glucose. A concentration range of standard D-glucose solution with a concentration of 0.05 mg/mL was used to establish the standard curve for total sugars. The absorbance (optical density) was read at 492 nm with the BK-UV1000 spectrophotometer.

2.1.6 Mineral contents

The mineral composition of the samples was determined from the ash of each sample. The ashes obtained for each sample were placed in crucibles then added to ultrapure water containing 2% nitric acid. After digestion, the mixture was filtered into 50 ml Falcon tubes using millipore filter paper. The filtrates obtained were introduced by suction into a previously calibrated flame spectrophotometer which measures the optical densities of the different minerals. Mineral concentrations were deduced from the standard curves for each mineral.

2.2 Microbiological Analyzes

2.2.1 Preparation of diluent and decimal dilutions

The diluent was prepared by dissolving 9 g of sodium chloride in 1000 mL of distilled water (NaCl, 0.9%). This solution was used for the preparation of the stock suspension and decimal dilutions for seeding. The stock suspension of each sample was prepared by introducing 10 g of *toedo* candy into 90 mL of sterile diluent. The

stock suspension was homogenized and then used for cascade dilutions. With a syringe, 1 mL of the stock solution was taken and introduced into a tube containing 9 mL of diluent. The mixture obtained corresponds to the 10⁻² dilution. The following dilutions were obtained by proceeding in the same way.

2.2.2 Preparation of culture media

Culture media were prepared by dissolving an appropriate mass of each culture medium in distilled water according to the manufacturer's recommendations. These culture media were then placed in a water bath until completely dissolved and then sterilized at 121°C for 15 minutes in an autoclave. Each medium was cooled at around 45°C to 50°C and then poured into sterile Petri dishes.

2.2.3 Seeding and incubation

The surface seeding method was used. This technique consists of depositing a volume of inoculum (100 µL) of the appropriate dilution on the surface of the agar and spreading it over the latter using a rake-shaped Pasteur pipette. The dilutions retained were inoculated for each germ researched on the appropriate media. The seeded Petri dishes were inverted and placed in incubators set at different temperatures depending on the type of germ researched. Information concerning the culture media used, and the incubation conditions of the different germs researched are recorded in Table 1.

2.2.4 Expression of results

After the incubation period, the number of microorganisms was determined in the Petri dishes by colony counting. According to Standard ISO 4833-2 [13], the calculation was made taking into account the Petri dishes of two successive dilutions. When the number of colonies in these Petri dishes was between 15

and 300, the number of germs (N) was calculated according to the following formula:

$$N = \frac{\sum C}{V(n_1 + 0,1n_2)d}$$

N: represents the number of microorganisms (CFU/g);

ΣC: Sum of colonies counted on the Petri dishes retained from the two successive dilutions;

n1: Number of Petri dishes retained at the first dilution and n2 the number of Petri dishes retained at the second dilution;

d: First dilution retained;

V: volume of inoculum seeded in each Petri dish.

For Petri dishes that contained number of colonies less than 15, the following formula was used:

$$N = \frac{\sum C}{V * d * n}$$

For Petri dishes containing no colonies, the result was considered as follows: N<1/d

N = number of microorganisms in CFU/g of product

ΣC = number of colonies counted on the Petri dishes retained

V = Volume of inoculum

d = rate of dilution retained

n= number of Petri dishes used at the chosen dilution.

2.3 Statistical Analyzes

XLSTAT software version 2019 was used for the statistical analyzes of the data. Analyze of variance (ANOVA) was used to compare the different means of the physicochemical variables using the Newman-Keuls test at the probability threshold p = 0.05.

Table 1. Germs researched, culture media used and incubation conditions

Microorganisms	Culture media	References	Temperature and incubation time
TMAF	PCA	ISO4833 [10]	37°C for 24h
TC	EMB	ISO4832 [11]	37°C for 24h
TTC	EMB	ISO4832 [11]	44°C for 24h
YM	SCA	NF V08-059 [12]	30°C for 3 to 5 days

TMAF : Total Mesophilic Aerobic Flora, TC : Total Coliforms, TTC : Thermotolerant Coliforms, PCA : Plate Count Agar, EMB : Eosin Methylene Blue Agar, SCA : Sabouraud Chloramphenicol Agar, h : hour

3. RESULTS AND DISCUSSION

3.1 Physico-chemical Characteristics of *Toedo* Candies

The physicochemical characteristics of the samples analyzed are recorded in Table 2. The values of the different parameters varied from one sample to another. Statistical analysis revealed a significant difference between the mean values of pH ($p=0.012$), titratable acidity ($p=0.000$), water content (0.000), dry matter content (0.000) and ash content (0.000) of the different samples. However, there was no significant difference between the mean values of Total sugars content ($p=0.445$).

3.2 Dry Matter

The dry matter varies from $84.04\pm0.14\%$ for MG2 to $96.16\pm0.00\%$ for N3 (Table 2). In general, the dry matter of the studied candies are higher than those recorded by Cebin et al. [14]. According to these authors, the dry matter of the candy formulations ranged approximately from 70 to 76%. The values obtained during this study for plain *toedo* candies and ginger *toedo* candies were higher than the average value obtained by Pagadjovongo [15] on *toedo* powder which was $88.89\pm0.13\%$. The high dry matter content of the samples shows that they could be stored for a long time. On the other hand, the dry matter contents of mango and ginger *toedo* candies were lower than the value obtained by Pagadjovongo [15]. This could be explained by the composition of the different *toedo* candies.

3.3 Water Content

The water content varies from $3.84\pm0.00\%$ (N1) to $16.06\pm0.14\%$ (MG2) (Table 2). The water contents of the different samples of *toedo* candy are generally lower than the water contents of the *toedo* powder reported by Assogbadio [16] which varied from 6 to 28%. *Toedo* mango and ginger candies have high water contents. These high levels of water could be explained by the presence of mango pulp. The variations in the water contents of the different samples could be explained by the intensity of drying applied by the different producers. The moisture content of candies is a very important quality parameter, as it majorly affects texture and the overall stability of the product, including microbial contamination [17].

3.4 Ash Content

The ash content of the samples varied from $1.30\pm0.21\%$ for MG1 to $1.90\pm0.06\%$ for N1 (Table 2). Mango and ginger *toedo* candies had the lowest ash contents, while plain *toedo* candies had the highest ash contents. The ash contents of the 9 samples were similar to those revealed by Santos et al. [18] which ranged from 1.38 to 1.83% in four brands of peanut-bases candies. However, the ash content recorded in this study are higher than the ash contents of *toedo* powder reported by Tapsoba [19] which ranged from 0.40% to 1.18%. On the other hand, the ash contents of the 9 samples were lower than that obtained by Joël et al. [5] which was 5.1%. The variation in ash content of *toedo* candies could be explained by the composition of the candies and the type of *toedo* powder used.

Table 2. Physico-chemical characteristics of *toedo* candies

S	WC (%)	DM (%)	AC (%)	TA (%)	pH	TSC (%)
N1	09.54 ± 0.08^d	90.4 ± 0.08^d	1.90 ± 0.06^a	0.164 ± 0.000^{bc}	3.89 ± 0.2^{ab}	53.78 ± 1.82^a
N2	11.81 ± 1.06^c	88.94 ± 1.06^e	1.52 ± 0.03^d	0.134 ± 0.002^e	3.90 ± 0.2^{ab}	55.44 ± 0.04^a
N3	03.84 ± 0.00^g	96.16 ± 0.00^a	1.72 ± 0.03^d	0.162 ± 0.002^c	3.89 ± 0.2^{ab}	51.53 ± 4.44^a
G1	08.53 ± 0.18^e	91.34 ± 0.18^c	1.81 ± 0.01^{ab}	0.166 ± 0.001^{ab}	3.88 ± 0.2^{ab}	52.38 ± 1.82^a
G2	05.61 ± 0.15^f	94.5 ± 0.16^b	1.66 ± 0.11^c	0.152 ± 0.001^d	3.91 ± 0.1^{ab}	53.04 ± 2.11^a
G3	09.76 ± 0.62^d	90.68 ± 0.62^d	1.48 ± 0.03^d	0.168 ± 0.002^a	3.87 ± 0.1^{ab}	55.07 ± 1.72^a
MG1	11.88 ± 0.16^c	88.24 ± 0.17^e	1.3 ± 0.21^e	0.133 ± 0.000^e	3.90 ± 0.1^a	53.32 ± 2.85^a
MG2	16.06 ± 0.14^a	84.04 ± 0.14^g	1.34 ± 0.06^e	0.132 ± 0.001^e	3.90 ± 0.1^a	54.08 ± 0.24^a
MG3	14.87 ± 0.89^b	84.5 ± 0.89^f	1.59 ± 0.13^{cd}	0.131 ± 0.001^e	3.90 ± 0.1^{ab}	54.76 ± 0.22^a
Pr>F	0.0001	0.0001	0.0001	0.0001	0.012	0.445
Significant	Yes	Yes	Yes	Yes	Yes	No

In the same column, the values bearing the same letters are not significantly different according to the Newman-Keul test at the threshold of $P = 0.05$.

S : Sample, WC: Water content, DM : Dry matter, AC: Ash content, TA: Titratable acidity, TSC : Total sugars content

3.5 pH

The pH of the samples varied from 3.87 ± 0.1 for G3 to 3.90 ± 0.1 for MG1 and MG2 (Table 2). These values were higher than those obtained by Joël et al. [5] and Pamba et al. [20] on *teodo* powder which were 3.30 and 3.29 respectively. However, they are similar to those obtained from different formulations of gummy candies [14]. The pH values of the different samples show that *teodo* candies are acidic. Thus, the *teodo* candies used during this study could be easily stored for a long time.

3.6 Titratable Acidity

The titratable acidity of the samples varied from $0.131 \pm 0.001\%$ for MG3 to $0.168 \pm 0.002\%$ for G3 (Table 2). Low titratable acidity values are achieved with mango and ginger *teodo* candies. The titratable acidity of the 9 samples was higher than that reported by Joël et al. [5] on *teodo* powder which was 0.102%. A high titratable acidity value would allow better conservation of *teodo* candies.

3.7 Total Sugar Content

The 9 samples of *teodo* candy presented relatively different Total sugars contents. However, the analyzes of variance showed that there is no significant difference between the sugars contents of the different samples. These contents varied from 51.53% (N3) to 55.53% (N2). The sugars contents contents obtained during this study were generally lower than those obtained by Martin [21] on *teodo* powder which varied from 50 to 55%. However, the sugars contents of all the samples analyzed were higher than that reported by Pamba et al. [20] which was 20.12%. Similarly, Santos et al. [18] obtained lower carbohydrate levels than those

obtained during this from peanut-based candies named paçoca, ranging from to 32.98% to 46.74%. The variability in carbohydrate contents could be explained by the degree of maturity of the fruits used and their origins.

3.8 Mineral Content

The mineral contents of the different samples are recorded in Table 3.

The mineral contents of the 9 samples varied from one sample to another. Indeed, the iron content varied from 49.30mg/100g (MG2) to 129.25mg/100g (N1); the Zinc content, from 1.37mg/100g (MG2) to 14.92mg/100g (G1); the calcium content, from 17.60mg/100g (MG1) to 43.00mg/100g (MG3) and the magnesium content, from 3.53mg/100g to 42.38mg/100g. These samples had lower calcium and zinc contents than those obtained by Joël et al. [5]. This author reported calcium and zinc contents of 293mg/100g and 21mg/100g respectively in *teodo* powder. However, the iron and magnesium contents were higher than those obtained by Joël et al. [5] which were 76mg/100g and 2.12mg/100g respectively. The calcium and magnesium contents of the samples studied were all lower than those reported by Pamba et al. [20] which were 231.21 mg/100g and 77.69mg/100g respectively. However, the iron and zinc contents of all samples were higher than those reported by Pamba et al. [20] which were 6.31 mg/100g and 1.2mg/100g respectively.

3.9 Microbiological Characteristics of *Toedo* Candies

The microbiological loads of the *teodo* candy samples are reported in Table 4.

Table 3. Mineral content of *teodo* candy samples

Sample	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)
N1	59.50	6.44	43.30	38.25
N2	49.95	5.77	41.00	20.99
N3	104.90	9.73	26.80	23.06
G1	93.30	14.92	33.50	42.38
G2	129.25	12.25	28.20	16.14
G3	87.65	7.80	28.40	29.70
MG1	68.60	10.82	17.60	12.03
MG2	49.30	1.37	20.00	3.53
MG3	127.50	11.26	43.00	15.14

Table 4. Microbiological characteristics of *toedo* candy samples

Sample	TMAF	YM	TC	TTC	Appreciation
N1	2.10×10^4	10^2	5×10^2	2×10^2	Unsatisfactory
N2	2.90×10^4	<10	<10	<10	Satisfactory
N3	2.50×10^4	<10	10^2	<10	Acceptable
G1	4.90×10^4	<10	<10	<10	Satisfactory
G2	3.80×10^4	10^2	10^2	<10	Acceptable
G3	2.50×10^3	<10	10^2	<10	Acceptable
MG1	2.20×10^3	2×10^2	<10	<10	Satisfactory
MG2	6.10×10^3	2×10^2	<10	<10	Satisfactory
MG3	5.20×10^3	<10	10^2	<10	Acceptable

TMAF: Total Mesophilic Aerobic Flora, YM: Yeasts and molds, TC: Total coliforms, TTC: Thermotolerant coliforms.

3.10 Total Mesophilic Aerobic Flora

The total mesophilic aerobic flora load of the samples varied from 2.20×10^3 (MG1) to 4.90×10^4 CFU/g. The loads of TMAF recorded in this study are similar those obtained by Santos et al. [18] which ranged from $7,00 \times 10^1$ to 4.4×10^3 CFU/g in peanut-based candies. For samples MG1 and G3, the TMAF loads were lower than that obtained by Cissé et al. [22] for *toedo* powder which was 3.70×10^3 CFU/g. On the other hand, for the other samples, the loadings are higher than that obtained by Cissé et al. [22]. Samples G3 and MG1 had TMAF loads lower than that obtained by Pamba et al. [20] which was 4.70×10^3 CFU/g but the other samples had loads higher than that reported by Pamba et al. [20] for this type of germ. Thus, according to the interpretation guidelines of the Government of Luxembourg [23], all the *toedo* candy samples were of satisfactory quality for this type of germ. These results could be explained by compliance with good hygiene and manufacturing practices. In addition, the acidic pH and low water contents of the samples could explain their low TMAF loadings.

3.11 Yeasts and Molds

The yeast and mold loads of the samples were less than 10 CFU/g for samples N2, N3, G1, G3 and MG3, 10^2 CFU/g for N1 and G2 and 2.00×10^2 CFU/g for MG1 and MG2. According to the interpretation guidelines of the Government of Luxembourg [23], all samples analyzed were of satisfactory quality. For samples MG1 and MG2, the loads obtained were higher than that reported by Diop et al. [21] which was 10^2 CFU/g for *toedo* powder. On the other hand, samples N2, N3, G1, G3 and MG3 presented loads lower than that of Diop et al. [24]. While the loads of samples G1 and G2 were similar to that obtained

by Diop et al. [24]. The yeast and mold loads of all samples were lower than the load reported by Pamba et al. [20] which was 2.00×10^3 CFU/g. As the samples analyzed were all of satisfactory quality, this could be explained by compliance with good hygiene and manufacturing practices. The low yeast and mold loads could allow better conservation of *toedo* candies.

3.12 Total and Thermotolerant Coliforms

The total coliform loads of the samples are presented in Table 3. For N1 the load was 5.00×10^2 CFU/g. For each of the samples N3, G2, G3 and MG3 the load was 10^2 CFU/g. For the other samples the loads were less than 10 CFU/g. For most samples, the thermotolerant coliform loads were less than 10 CFU/g, only sample N1 had a load of 2.00×10^2 CFU/g. According to the guidelines for the interpretation of the Government of Luxembourg [23] concerning snack products and fruit powders, samples N2, N3, G1, G2, G3, MG1, MG2 and MG3 were of satisfactory quality for thermotolerant coliforms but sample N1 was of unsatisfactory quality. For total coliforms, samples N2, G1, MG1 and MG2 were of satisfactory quality according to the guidelines for the interpretation of the Government of Luxembourg [23] which recommends a load of between 10^2 and 10^3 CFU/g, on the other hand the other samples were of acceptable quality for this type of germ.

In sum, for all the germs studied, 11.11% (1/9) of the samples were of unsatisfactory quality, 44.44% (4/9) of the samples were of acceptable quality and 44.44% (4/9) samples were of satisfactory quality. The mango and ginger *toedo* candies analyzed presented better microbiological quality despite their high water contents, this could be explained by the respect

of good hygiene practices during the production of this type of candy. It is therefore important to raise awareness among *teodo* candy producers in the city of Ouagadougou and train them in good hygiene and manufacturing practices in order to further improve the microbiological quality of *teodo* candy.

4. CONCLUSION

This study focused on *teodo* candies, produced and consumed in Burkina Faso. At the end of the study, it should be noted that *teodo* candies present variable physicochemical characteristics in general and are acidic. These candies contain appreciable amounts of minerals like iron, zinc, calcium and magnesium. Microbiological analyze shows that most samples contain low loads of FAMT and yeasts and molds. Infotunately, some samples contain coliforms. The study shows that the majority of samples were of acceptable or unsatisfactory microbiological quality. Thus, it would be beneficial to take steps to improve the quality of *teodo* candies for consumers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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