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Physicochemical Characteristics of Curdled Milk and Their Correlation with the Lactic Flora in Chad

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Abstract

Milk and dairy products are highly consumed in Chad. Among dairy products, curd is very popular chadian consumers. In order to determine the values of various physicochemical parameters of curds, but also the relationships between these parameters and the lactic flora, a study was conducted during two years (2006 and 2008) in cold dry season and hot dry season. In total five locations was study during these two seasons. It was noted that there is a very significant difference between physicochemical parameters ($P<0.005$). Also, fermenting milk with micro-organisms leads to changes in the texture, color, taste, flavor and nutritional properties of milk. Similarly, bacterial species are different from one locality to another, although some species have been identified in the curdled milk of all areas of study. Fermenting milk leads to the formation of organic acids, especially lactic acid, resulting in acidification of the milk. Production of lactic acid during the fermentation results in a lowering of pH will cause the milk to curdle. It is therefore important to conduct this study in order to capitalize information on the characteristics of the curd according to the different seasons. This study would be a good tool for information and awareness on the quality of curd in Chad. It would be desirable that other much more sustained studies be carried out to identify the majority of lactic acid bacteria. This will allow better synergies in order to present for consumers the various curds of best organoleptic characteristic.

Key words: Milk curds, Physicochemical Parameters, Lactic flora

1. Introduction

Milk is a nutritious food for humans. In Chad, milk and its products are consumed by many people including the curd its in various forms (Doutoum et *al*., 2013a; 2013b); it is favorable to the growth of many microorganisms particularly pathogenic bacteria (Chye et al., 2004). It should also be noted that the composition and physico-chemical characteristics of milk varies significantly between species and even between races (Soryal et *al*., 2004). These characteristics are also dependent on the nature of the feed (Sutton, 1989; Coulon et *al*., 1995.). Dairy products are considered nutrient-rich foods. They provide with grains, meats, vegetables and fruits, many nutrients in relatively low energy and essential to health throughout the life cycle (Drewnowski, 2005; Miller, Jarvis McBean, 2007). The processing of milk into dairy products with an economic life and greater conservation developed in dairy processing units. Koussou (2001) & Duteurtre et *al*. (2005) indicates that in Chad, there is a renewed interest in local milk and traditional products such as fresh milk, fermented milk, clarified butter, which represented up to 65% of dairy products sold in major cities. In addition to their nutritional value, consumption of dairy products is associated with beneficial health effects (Takahiro et *al*., 2007). In Chad, the traditional milk curds are made in all suburban areas. These dairy products are very popular with consumers because of their ease of assimilation but also because of their organoleptic characteristics. Unfortunately some physicochemical parameters (pH, temperature, sample temperature, Dornic acidity and relative humidity) when not controlled distort curds or if not make them inedible. Therefore, this study was conducted in five locations for two seasons in order to assess the effect of these parameters on the quality of curds. The general objective of this work is to know the direct role of these parameters on the curds in different localities. Specifically this study aims to identify the relationship between seasonal and regional variations of curds and lactic flora present in these products.

2. Materials and Methods

Work was carried out at the Laboratory of Veterinary Research and Zootechnical of Farcha in Chad to Health and Food Industry Services Foodstuffs of Animal Origin.

2.1 Areas of sampling

Five locations were selected for this study. This is N'Djamena, Sarh, Moundou, Abeche and Bongor (Figure 1). These areas were chosen based on animal concentration in the dry season but also depending on the development of businesses related to the dairy industry.

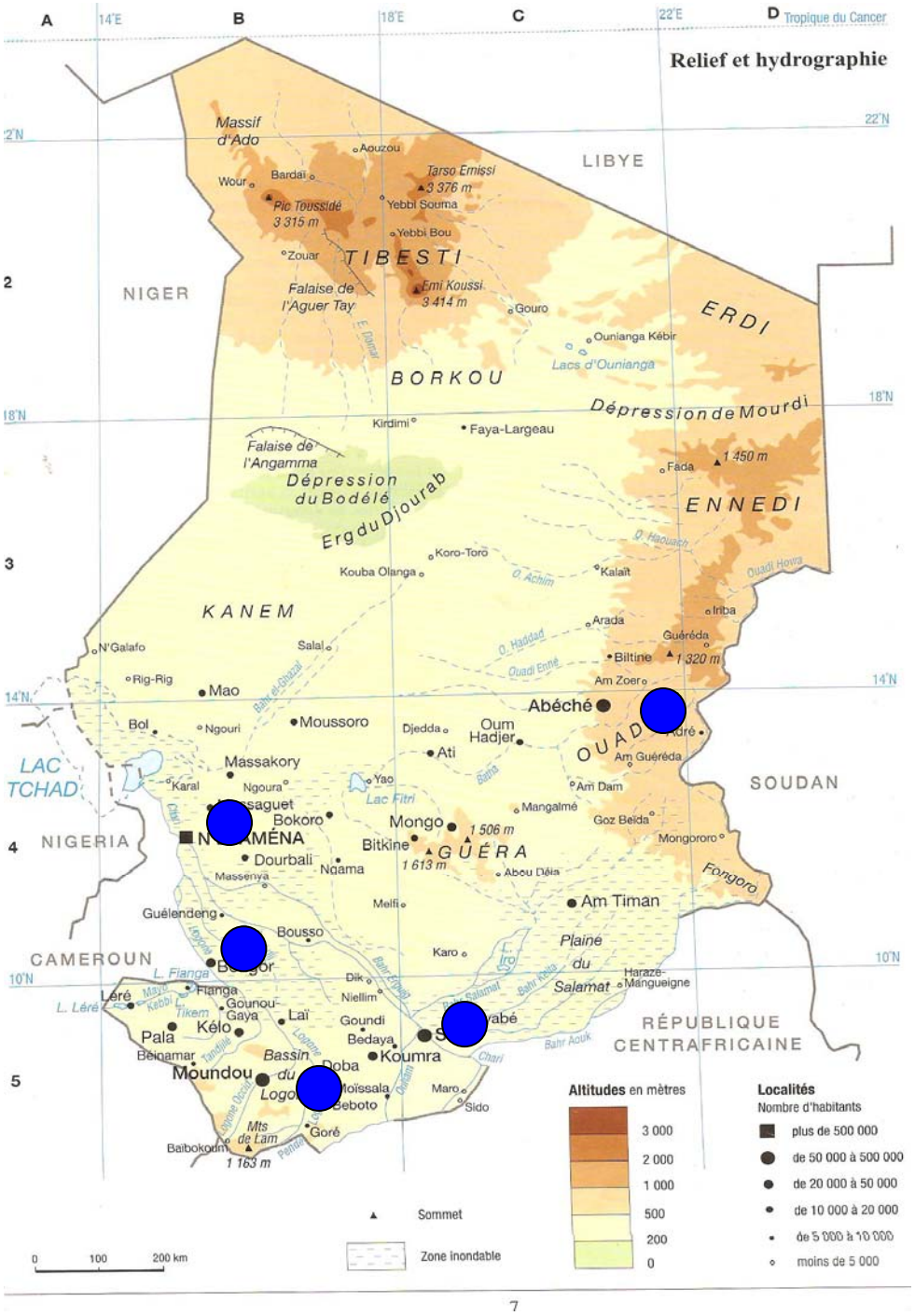


Figure 1. Location of study areas indicated by blue circles (Source: Jeune Afrique, 2006 amended)

2.2 Sampling and equipment

As Chad has several agro-ecological regions, it was necessary to have a representative in the quantity and quality of production areas curd samples. And for a period of two years, one thousand (1000) samples were taken at random, five hundred (500) during the cold dry

season (October to December) and five hundred (500) during the hot dry season (March-May) in the same sites.

2.3 Sampling method

Samples of curds were collected as aseptic rules respecting rigorous hygiene standards. It consists of plastic pots of 250 ml volume. By producer we take 10 samples of 250 ml who all come from different cans of 40 liters. Identifications are made in indelible ink. This is to avoid sampling the curd having the same origin, or at least whose manufacturing practices are similar. Quickly, we introduce samples into the cooler to prevent microbial growth. Thus the chain is maintained to the storage location. This continues until one hundred samples suburban area. After sampling, the samples were quickly introduced into a cooler with bottles of dry ice and crushed ice to maintain samples at very low temperatures. We used as thermo-hygrometers and pH meter probe to allow us to measure the temperature, moisture and pH levels. All samples are kept in cold rooms at the Veterinary and Zoo Technical Research Laboratory of Farcha (LRVZ).

2.4 Method of analysis in the laboratory

Once the samples arrive, they are introduced into the cold rooms of the laboratory. But the next day we take to determine the Dornic acidity or titration. For this we take a volume of 10 ml curd is placed in a beaker, and then adds 2 to 3 drops of phenolphthalein 1%. Then the beaker was shaken to homogenize the mixture. It added dropwise sodium hydroxide contained in the burette until the color changes from white to pink. The color should persist for at least 8 seconds. Reading the burette drop is made. We expressed the results Dornic degree (° D) multiplying the result by 10. The equipment used includes freezers and refrigerators, slide, balance precision weighing, test tubes, Petri dishes, bottles of 500ml, pipettes of 1, 2, 3, 5 and 10 ml, hood laminar flow for sterilization ovens, water baths, autoclaves to sterilize culture media player colonies. Culture media are de Man Rogosa and Sharpe (MRS) and M_{17} broth, agar and glassware. They are rarely used except for research of lactic acid bacteria community. They were ordered from Spain (Barcelona). They are very selective with the exception of some spoilage bacteria that can grow there (*Escherichia coli*) environments. We have prepared four culture media, two plates and two broths. These are MRS and M_{17}. The method of preparation is the same. After weighing the medium in the form of powder and determined the amount of distilled water, the mixture is introduced into a flask containing a little distilled water. Then the remainder of distilled water required is completed. We stir the bottle to mix the contents of the vial. Then the mixture is heated in a pot. When everything becomes homogeneous, the medium is sterilized in an autoclave. After that, it is cooled and placed in a water bath before use. By cons broth after cooling is kept in the refrigerator.

2.5 Material of identification and confirmation

For the different identifications of lactic acid bacteria, we used two types of galleries: Galerie API 20 STREP and API 50 CH gallery-L with their reagent kit. For confirmation we used catalogs identification. The software web api TM was used.

2.6 Research Methods of lactic acid bacteria

All samples studied have undergone preliminary treatment to obtain dilutions according to standard NF V08 -010 (March 1996). We introduce in STOMACHERND bag 10 ml of the sample to which are added 90 ml of MRS broth or broth or M_{17}. Lactic flora was enumerated according to the NF ISO 15214 (September 1998). The culture media used are: broth and agar MRS for the enumeration of lactobacilli and M_{17} medium for the enumeration of streptococci. For each sample, the morphological characteristics of the colonies (size, shape, color) are observed. Lactobacillus colonies are round, lenticular, vary in size (1-4 mm), those of Streptococcus are round or lenticular, with regular contours, a white opaque.

2.7 Identification and confirmation method (NF ISO 15214, September 1998)

We must clarify that the wells after receiving tabs reagents are covered by a plastic cover to prevent contamination. For bacteria grown in MRS agar, we use the API 50 CH galleries -L. On the cup with 50 stalls, we put a small amount of distilled water to be able to fix the plates that have compartments containing chemicals that are capable of reacting with the bacterial suspension with or without color change water. Indeed, it there's a note that explains the positivity or negativity of a reaction depending on the color and tell us the number to write. And so we get a code number and we will consult the grid for the name of the bacterium with a percentage of identification and testing against. For bacteria grown in M_{17} agar, the cup is more simplified and has only ten cubicles allowing the strip containing the reagents to adhere by putting a small amount of distilled water. Each stall is filled with the suspension is at half or three-quarters full. Some stalls are filled entirely from the paraffin oil for achieving anaerobiosis. For the M_{17}, there's a reading that it takes 2 hours and another 24 h. MRS reading is at 24 h only. After reading the bacteria are identified. But to avoid any confusion the APIWEB TM software is used for the second time with the same code.

2.8 Statistical Analysis

The collected data are entered in the spreadsheet Microsoft Excel version 2003. Then to compare means, we used the SPSS 16. 0 software from WINDOWS including Student's t test and analysis of variance (ANOVA) with a factor.

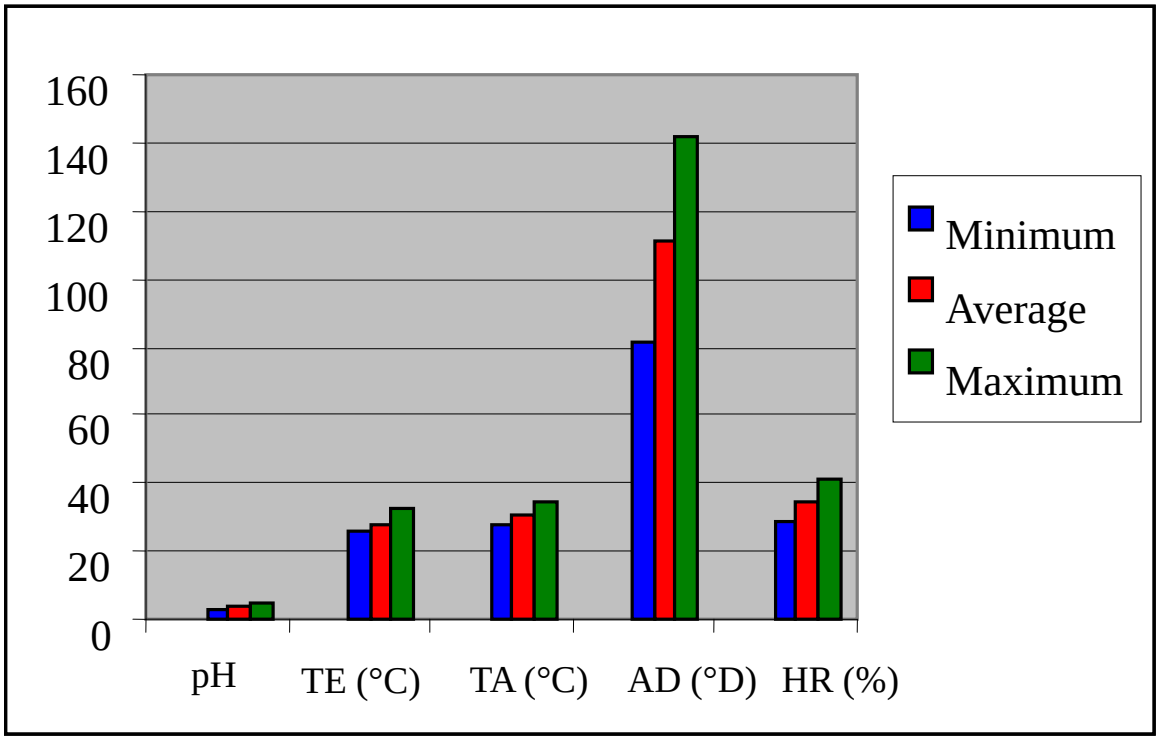
3. Results

3.1 Locality of Abeche

Table 1. Values of physicochemical parameters obtained in 2006 and 2008

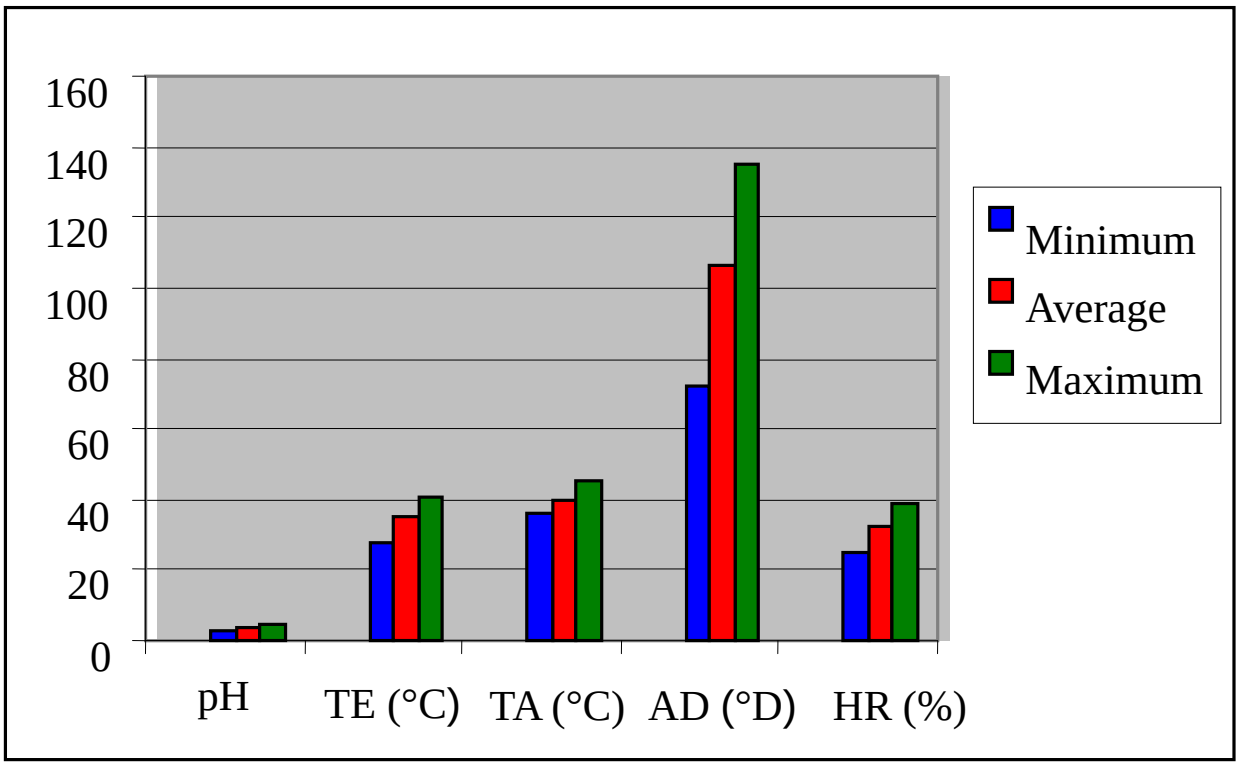
Values / Parameters	Minimum		Maximum		Average		Comparison test	
	CDS	HDS	CDS	HDS	CDS	HDS	P	Signif
pH	3	3. 10	4. 91	5. 00	3. 79	3. 77	0. 639	*
Sample Temp (° C)	26	27. 32	32. 33	40. 87	28.15	35. 19	0. 000	***
Ambient Temp (° C)	27. 34	36. 05	34. 53	44. 99	30. 96	40. 06	0. 000	***
Dornic acidity (° D)	81	72	142	135	111. 27	106. 51	0. 003	**
Humidity (%)	29	25	41	39	34. 86	31. 93	0. 000	***

CDS = Cold Dry Season; HDS = Hot Dry Season; Signif = signification; * = Not significant; ** = Signifiant; ** = Very Signifiant



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

Figure 2. Physicochemical parameters of the town of Bongor on cold dry season



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

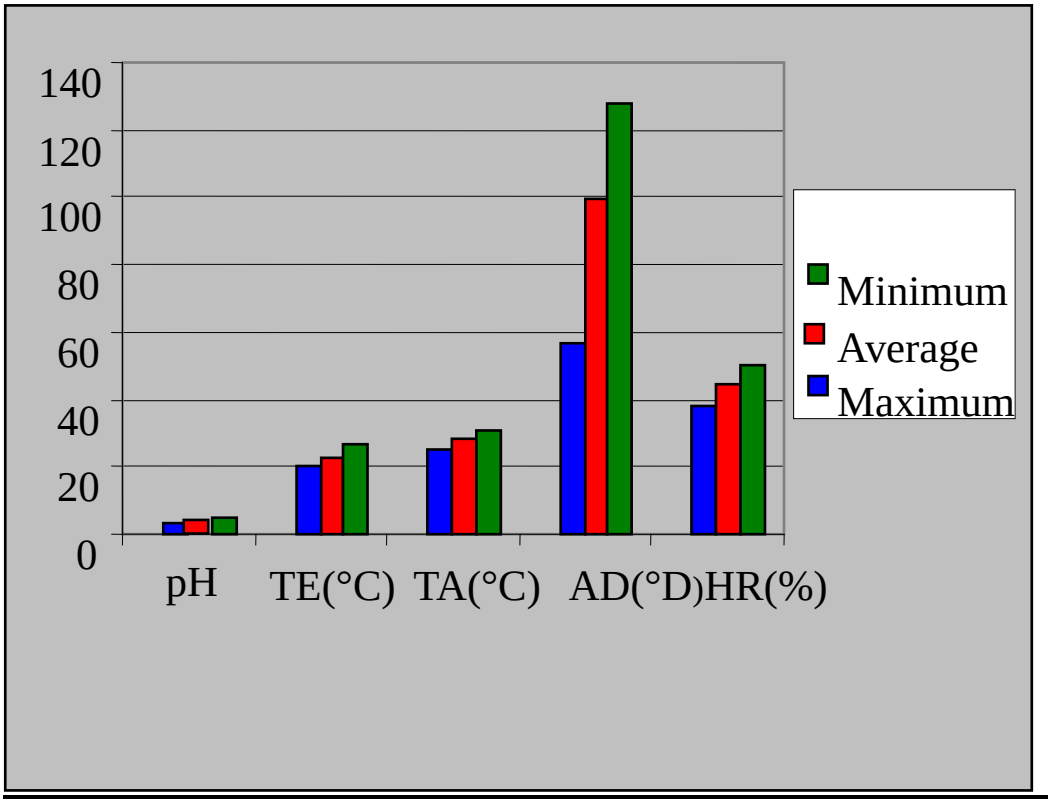
Figure 3. Physicochemical parameters of the town of Bongor on hot dry season

3.2 Locality of N'Djamena

Table 2. Values of physicochemical parameters obtained in 2006 and 2008

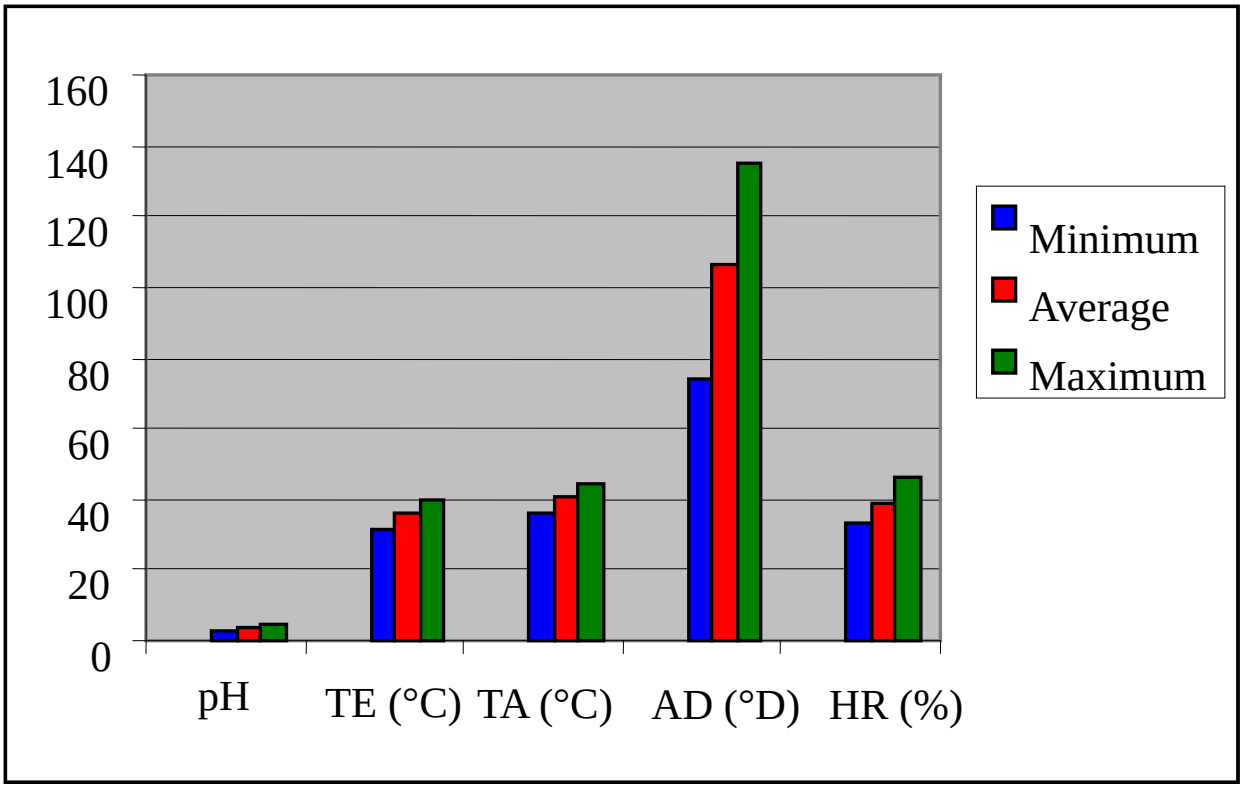
Values / Parameters	Minimum		Maximum		Average		Comparison test	
	CDS	HDS	CDS	HDS	CDS	HDS	P	Signif
pH	3. 13	3. 00	4. 56	5. 00	3.92	3.75	0. 000	***
Sample Temp (°C)	20. 08	31. 09	27. 00	39. 97	22.81	35. 86	0. 000	***
Ambient Temp (°C)	25. 10	36. 01	30. 94	44. 71	28.00	40.56	0. 000	***
Dornic Acidity(°D)	57	74	128	135	99.75	106.45	0. 000	***
Humidity (%)	38	33	50	46	44.16	38.93	0. 000	***

CDS = Cold Dry Season; HDS = Hot Dry Season; Signif = signification; * = Not significant; ** = Signifiant; ** = Very signifiant



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

Figure 4. Physicochemical parameters of the town of N'Djamena on cold dry season



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

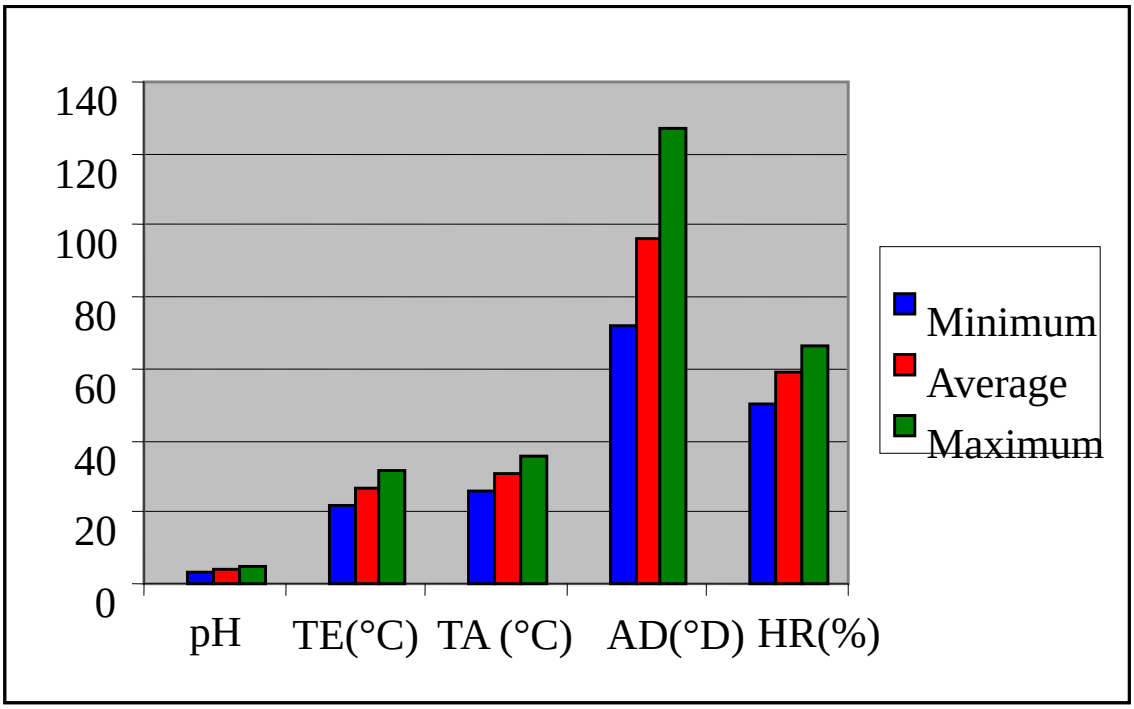
Figure 5. Physicochemical parameters of the town of N'Djamena on hot dry season

3.3 Locality of Bongor

Table 3: Values of physicochemical parameters obtained in 2006 and 2008

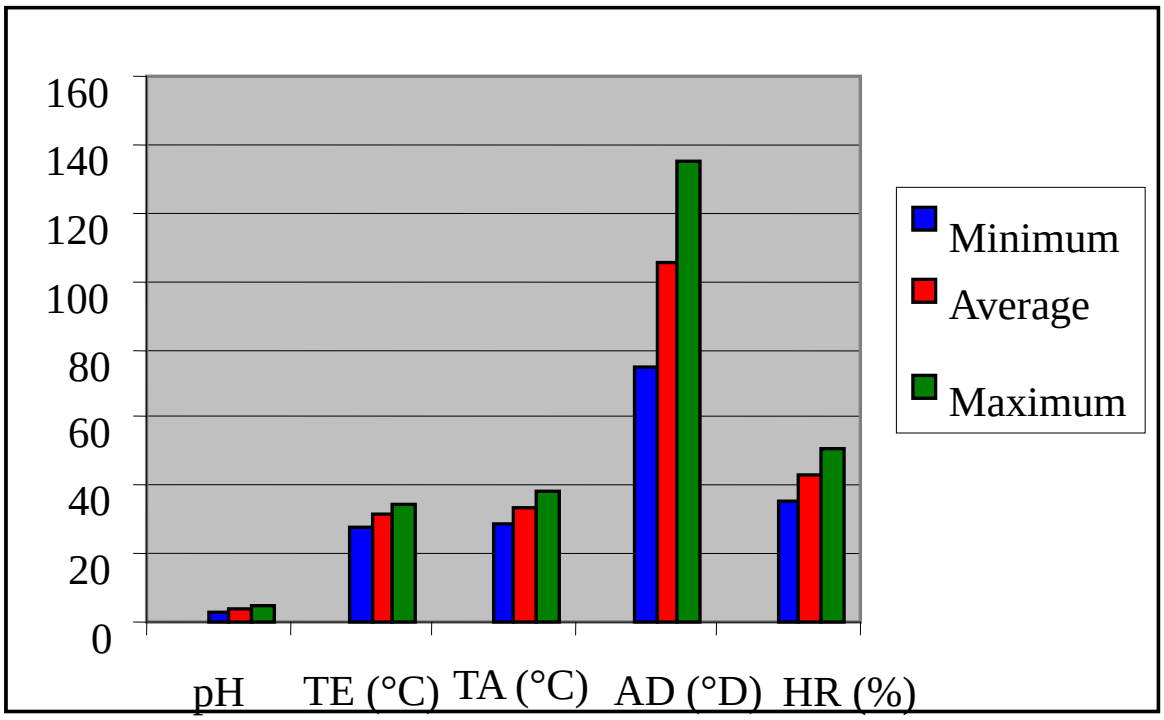
Values / Parameters	Minimum		Maximum		Average		Comparison test	
	CDS	HDS	CDS	HDS	CDS	HDS	P	signif
pH	3. 00	3. 38	5. 00	4. 91	3. 82	4. 07	0. 000	***
Sample Temp (°C)	28. 12	21. 70	34. 95	31. 20	31. 97	26. 33	0. 000	***
Ambient Temp (°C)	28. 40	26. 00	38. 42	35. 50	33. 37	30. 55	0. 000	***
Dornic Acidity (°D)	75	72	135	127	105. 27	96. 34	0. 000	***
Humidity (%)	35	50	51	66	42. 95	59. 06	0. 000	***

CDS = Cold Dry Season; HDS = Hot Dry Season; Signif = signification; * = Not significant; ** = Significant; ** = Very Signifiant



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

Figure 6. Physicochemical parameters of the town of Bongor on cold dry season



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

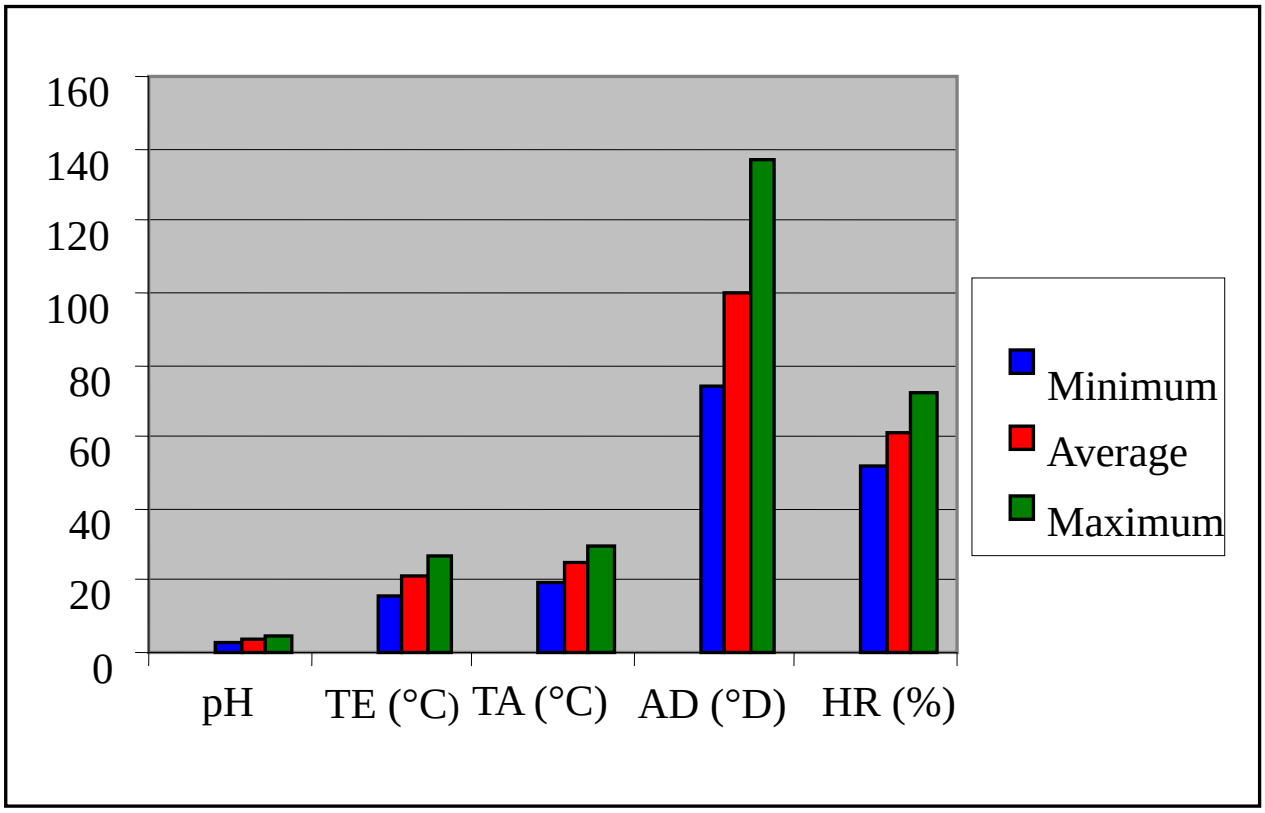
Figure 7. Physicochemical parameters of the town of N'Djamena on hot dry season

3.4 Location of Sarh

Table 4. Values of physicochemical parameters obtained in 2006 and 2008

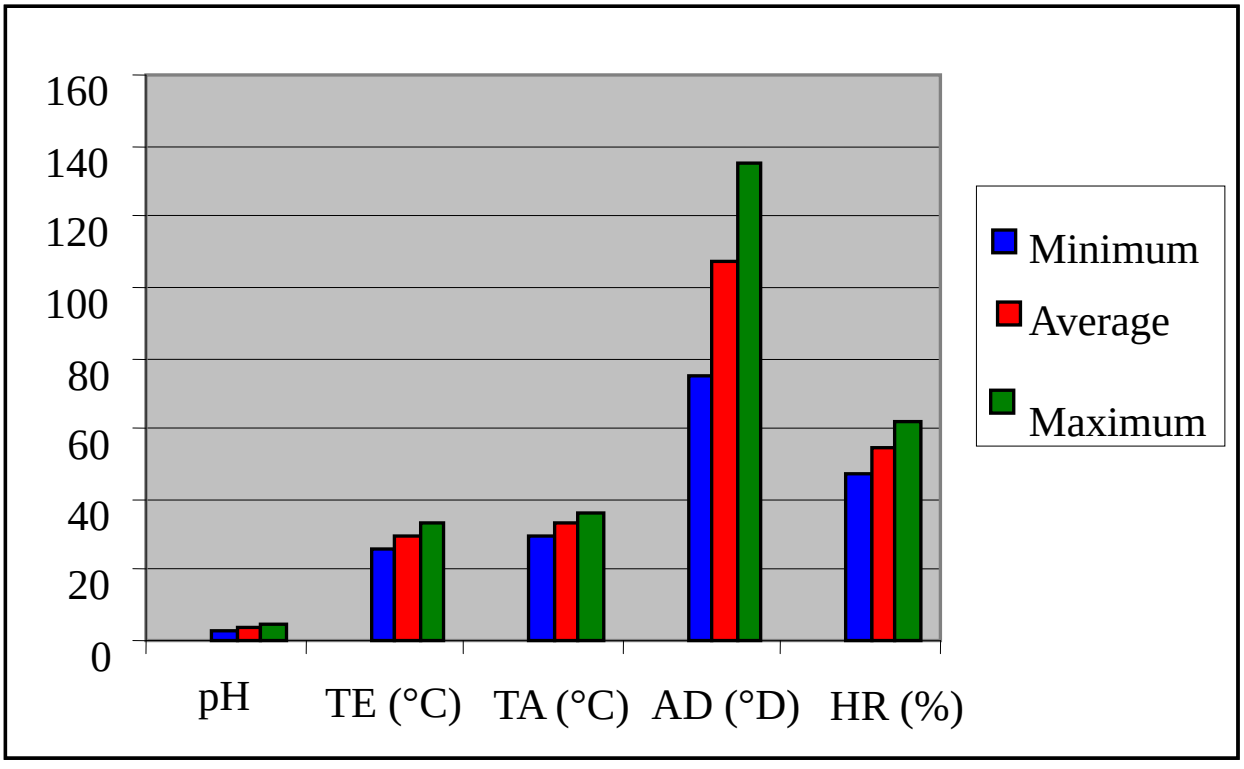
Values / Parameters	Minimum		Maximum		Average		Comparison test	
	DCS	HDS	CDS	HDS	CDS	HDS	P	Signif
pH	3. 12	3. 00	4,75	5. 00	4. 06	3. 79	0. 000	***
Sample Temp (°C)	16. 04	26. 12	26. 71	33. 44	19. 01	29. 91	0. 000	***
Ambient Temp (°C)	19. 01	29. 23	30. 02	36. 52	24. 77	33. 04	0. 000	***
Dornic Acidity(°D)	74	75	72	135	99. 58	107. 12	0. 000	***
Humidity (%)	52	47	135	62	60. 89	54. 87	0. 000	***

CDS = Cold Dry Season; HDS = Hot Dry Season; Signif = signification; * = Not significant; ** = Signifiant; ** = Very Signifiant



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

Figure 8. Physicochemical parameters of the town of Sarh on cold dry season



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

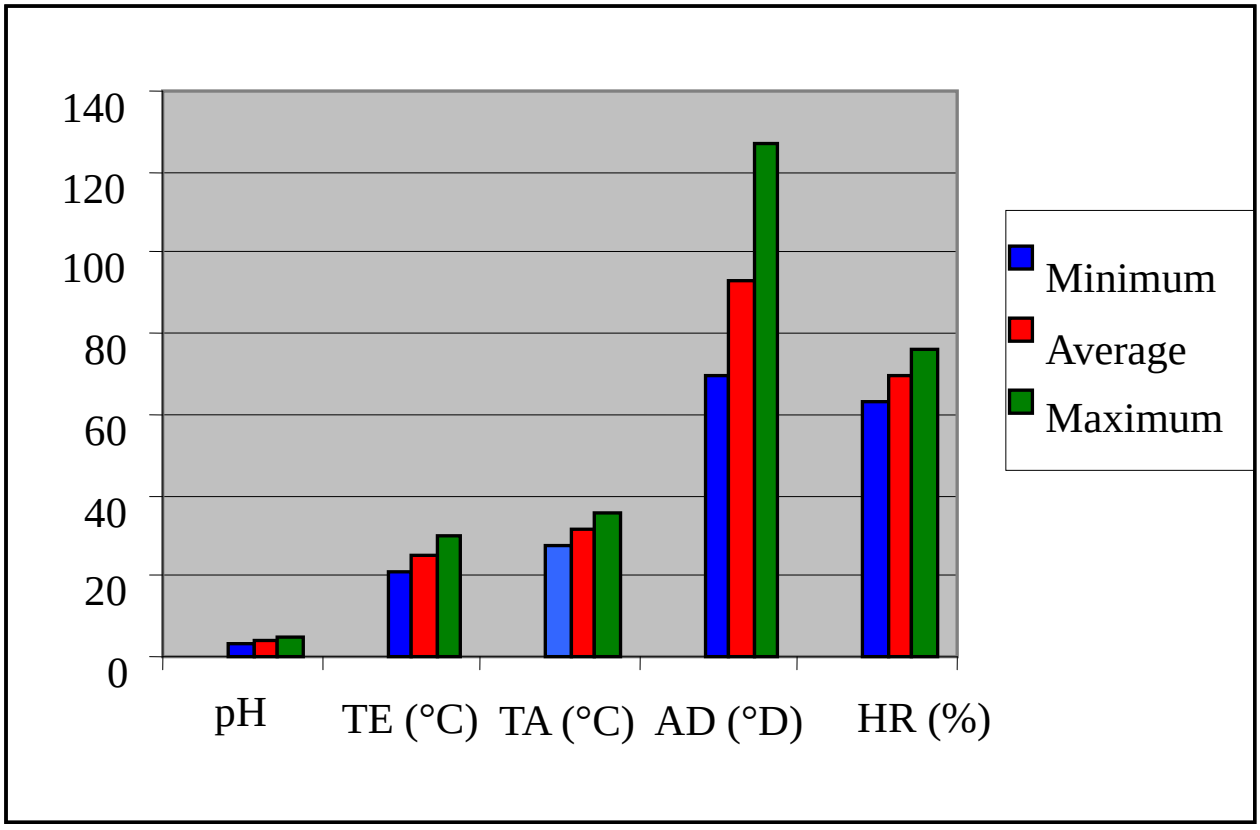
Figure 9. Physicochemical parameters of the town of Sarh on cold dry season

3.5 Locality of Moundou

Table 5. Values of physicochemical parameters obtained in 2006 and 2008

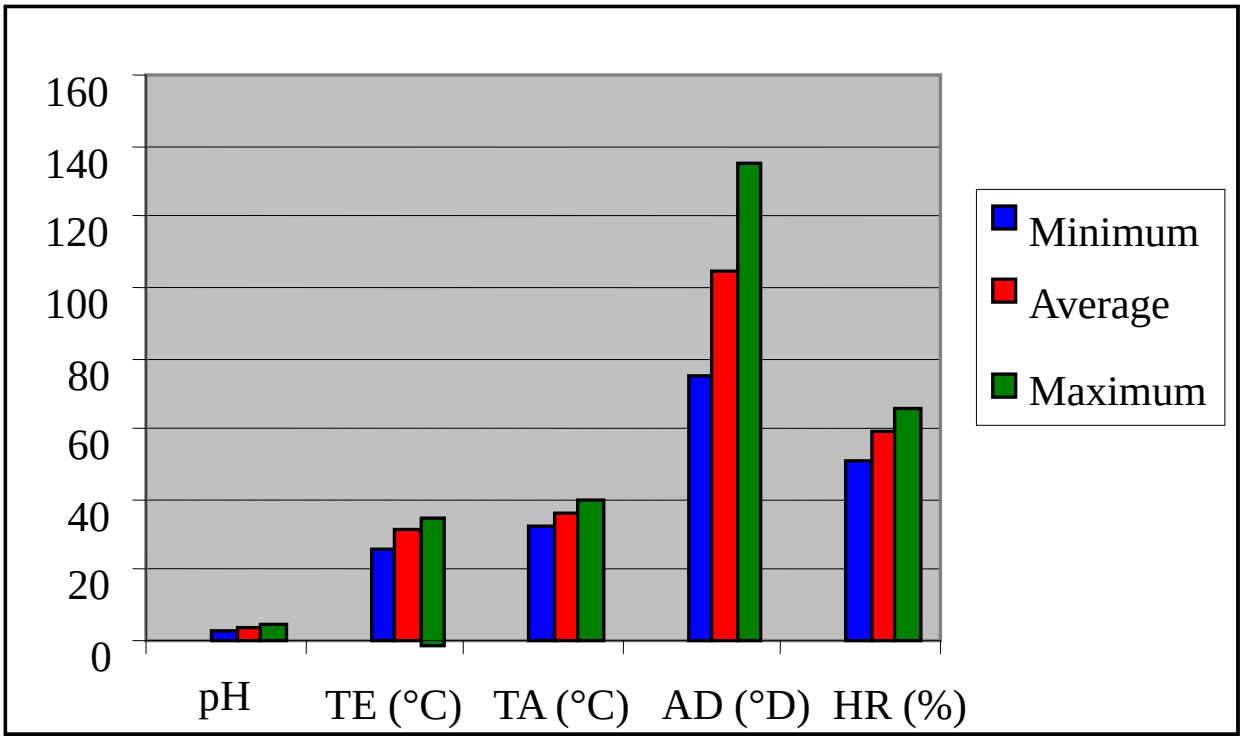
Values / Parameters	Minimum		Maximum		Average			
	CDS	HDS	SSF	CDS	HDS	CDS	P	Signif
pH	3. 37	3. 00	5. 00	5. 00	4. 19	3. 83	0. 000	***
Sample Temp (° C)	21. 35	26. 12	30. 00	36. 43	25. 33	31. 18	0. 000	***
Ambient Temp (° C)	27. 12	32. 09	35. 82	39. 71	31. 95	35. 87	0. 000	***
Dornic acidity (° D)	70	75	127	135	93. 02	104. 65	0. 000	***
Humidity (%)	63	51	76	66	69. 70	58. 77	0. 000	***

CDS = Cold Dry Season; HDS = Hot Dry Season; Signif = signification; * = Not significant; ** = Significant; ** = Very Significant



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

Figure 10. Physicochemical parameters of the town of Moundou on cold dry season



TE = Temperature of Sample; TA = Room Temperature; AD = Dornic acidity; HR = Relative Humidity

Figure 11. Physicochemical parameters of the town of Moundou on hot dry season

4. Discussion

We measured in our study, the following physico- chemical parameters of curds collected in Abeche, N'Djamena , Bongor Moundou and Sarh: pH, temperature of the sample, ambient temperature, relative humidity and acidity Dornic. Changes in the values of these parameters were also noted within the same locality in cold dry season and hot dry season between localities in the same seasons.

Compared to pH between the two seasons, it was noted slight differences in average in the town of Abeche of 3.79 against 3.77. The differences between the two seasons are not significant with P> 0.05. In the city of N'Djamena, the average pH was 3.92 against 3.75 during the hot dry season. These average values of pH between the two seasons for this locality have a very significant difference with $P < 0.01$. For the city of Bongor, average values are 3.82 and 4.07 for two seasons. There is a very significant difference between the two means with $P < 0.001$. At Sarh, where average values of 4.06 and 3.79, the difference between the two averages is highly significant with $P < 0.001$. The average pH is between seasons, 4.19 against 3.83 in Moundou. There is also a very significant difference between

the two means with P < 0.001. In all locations, the average pH readings are higher in cold dry season (3.79 to 4.19 °C) than in hot dry season (3.75 to 3.83 °C). This can be explained by the fact when it is very hot, the the pH decreases. Our results are lower than those obtained by Doutoum (1995) and are 4.11, but this author has worked on industrial milk curds. Our results are also lower than N'Diaye (1991), 4.17; Sina (1992) 4.75, Xantapoulos et *al*. (2006), 4.6 and Avila et *al*. (2005) 4. 33. These authors have also worked on industrial curd. Nko Sadi (2006) found a pH of curd at about 6.5. This pH is very high and can be explained by the fact that the measurements were made at the beginning of curdling. Chirica et *al*. (1998) reported that the optimum pH of lactic acid bacteria (*Streptococcus thermophilus*) is between 6 and 7. However it is rather pH of raw milk. But Fernandez et *al*. (2005) reported that another starter culture (*Lactobacillus bulgaricus*) grows at pH 2 to 3.7. He argues that all lactic acid bacteria do not grow at a very acidic pH. Bueno et *al*. (2006) obtained values varying pH but higher than ours (4.06, 4.02, 3.85) and that are optimal for the growth of lactic acid bacteria such as *Lactobacillus casei* and *Lactobacillus rhamnosus*. Sylla et *al*. (2005) found values close to ours with an average pH of 3.78. This compared to the yoghurt value is low (pH 4). Reflecting the excessive acidity of fermented milk can be explained first by the fermentation technique used in this controller. This high acidity can also be explained by the freshness of the raw material. To this end, Abdelgadir (1998) and other authors have shown that milk may have the same pH, so give the same stability in industrial processes and yet show significantly different acidities. Conversely, curds can have the same acidity and pH values; in this case, the observed differences are related to high solids content. Our results corroborate those of Dieng (2001), the study focused on industrial curdled milk: it found that 92% of the samples have a pH below 4.6. This pH inhibits the growth of pathogenic bacteria and ensures better preservation. Bouzaine et *al*. (2004) found different results from ours. Indeed they found a pH of 2 and it has a remarkable antimicrobial activity against pathogens and spoilage organisms. Yoon & Woon (2002) found an average pH of 3.5. They showed that bacteria such as *Lactobacillus acidophilus* and *Lactobacillus helveticus* fight against pathogenic bacteria. Bae & Lee (2002) obtained a pH higher (5.7) than ours and identified *Lactobacillus bulgaricus* including antibacterial activity. Ahn et *al*. (2002) reported very acidic pH (2.5) and identified *Lactobacillus acidophilus* which has activity against *Salmonella spp*, *Salmonella enteritidis* and *Salmonella typhimurium*. Kang et al. (2004) note that *Lactobacillus fermentum* multiplies at pH 2 to 4. Finally Ziadi et *al*. (2005) obtained a pH of 4.30, which is a value much higher than ours, this is explained by the fact that they have worked on industrial curd including control of the cold chain.

Indeed we know that the mean values of the sample temperature varies during the hot dry season, they range from 29.91 to 35.19 °C. With reference to the work of other authors, we can say that the pH values recorded during the hot dry season and cold dry season in all localities are conducive to the growth of lactic acid bacteria.

Regarding the sample temperature, the differences between the two means are very significant in all areas studied with P < 0.001. In all locations , we noted that the average temperature values of the samples are lower in cold dry season (22.81 °C to 28.19 °C) in hot dry season (29.91 °C to 35 , 19 °C). This can be explained by the fact that the temperature at

the reception of the sample is strongly influenced by the ambient temperature, the mean values for our study ranged from 24.71 ° C to 31.98 ° C in cold dry season and 33.31 ° C to 41.06 ° C during the hot dry season. The average values of temperatures of samples we have identified are not unique, since similar values were noted by other authors. This is the case Sylla et *al*. (2005) noted that 30 °C, Tahir (2007) recorded 29.14 °C, Hassane & Bremé (2007) noted 24 °C and Hadji & Yalinga (2007) reported 23.12 °C.

The average values of the ambient temperature have increased in all areas between the cold dry season (24.71 ° C to 31.98 °C) and the hot dry season (33.31 °C to 41.06 °C). These are normal values for these seasons in Chad. The differences are very significant $P < 0.001$. The ambient temperature has a direct effect on the temperature of the sample. We must say that the temperature of the milk is strongly influenced by the ambient temperature. It can go up to 45 ° C (ASECNA-Chad, 2008). Ambient temperature is 41.06 ° C in dry hot season against 30.96 in dry cold season. The maximum is 44.99 and the minimum is 36.05. This temperature rise can be explained by seasonal variation but also the location of the study area is semi-desert area. Ziadi et *al*. (2005) reported the same temperature but is control of the cold chain has to give the curd a satisfactory temperature.

Regarding the Dornic acidity, two lessons can be drawn from our analyzes . The first lesson is that in all localities except Abeche, the average values of the Dornic acidity are rated lower in cold dry season than in hot dry season. The second lesson is that in Abeche, the average values of the Dornic acidity is higher in cold dry season than in hot dry season and it is in this place that the highest value was observed (111.27 ° D). Several explanations can be given to these observations. First of all the variations within the same locality development from Abeche, in the two seasons can be explained by the fact that in hot dry season , the increase in temperature causes a decrease in pH and therefore an increase in acidity Dornic . This is the opinion of Sylla et *al*. (2005) who observed an average of 77 ° D and for them the samples to high acidity Doronic have a low pH. The elevation values of the Dornic acidity observed in Abeche in cold dry season and the hot dry season may be related to a high microbial activity. In other words, if acidity is higher, milk can be contaminated. Some authors have demonstrated this relationship. This is the case of N'Diaye (1991) who noted a medium acidity titration 152.6 ° D and showed that the curd was heavily contaminated with *Escherichia coli*, by yeasts and molds. This is also the case with Sina (1992) who reported values from 89 to 139 ° D with an absence of pathogenic flora but abundant flora alteration. For physico- chemical parameter variations were also observed between seasons within localities. So for the Abeche area the average value of the Dornic acidity increased during the two seasons from 111.27 ° ± 15.81 ° D to 106.5 ° D ± 16.29. For the locality of N' Djamena, average of about 99.75 ° ± 13.85 D increased to 106.45 ° D ± 16, 08. In the town of Bongor the average value in the cold season is 96.34 D ° ± 15.15.

The mean values of relative humidity in all localities are higher in cold dry season than in hot dry season. This is explained by the fact that the increase in temperature causes a decrease of humidity in the dry season. The recorded values are not exceptional because values similar to ours have been observed by other author: Tahir (2007) who's noted a value of 38%, Hassane & Bremé (2007) reported 46.27%, Sobdibé (2007) noted 62% and finally Hadj &Yalinga

reported a value of 72%. The high relative humidity tends to keep longer curd.

Conclusion

This pioneering study has identified lactic species. In general, the physico-chemical characteristics of the analyzed products are pretty good. The products are not too acidic and organoleptic qualities, texture and color are acceptable. However, parameters such as temperature and acidity Dornic have been changes with the seasons. The average values of temperatures of samples increases as well as the average values of ambient temperatures during the hot dry season. In cold dry season, the average values of the Dornic acidity are higher. It was also identified species of bacteria that differ from one locality to another, but *Streptococcus thermophilus, Lactococcus lactis diacetylactis, Lactococcus lactis cremoris, Lactococcus lactis lactis* are present in all the five localities. Perspective further research in other areas and during the rainy season and will contribute greatly enhance this topic. They may also provide researchers with information that will be used in particular in the context of research and development.

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