IJFANS
International Journal of Food And Nutritional Sciences

Volume 7, Issue 1, January 2018, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food and Nutritional Science

e-ISSN 2320 -7876 www.ijfans.com
Vol. 7, No. 1, January 2018

Research Paper ***Open Access***

FORMULATION AND ASSESSMENT OF PHYSICOCHEMICAL AND SENSORY PROPERTIES OF PROTEIN ENRICHED COOKIES

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Received on: 1st May, 2017 Accepted on: 25th October, 2017

The present study aimed at formulating cookies using composite flour with the benefit of less utilized yet nutritious millets. The physicochemical, shelf life and sensory properties of the cookies (soy+bajra+kodo millet flour) were studied. Cookies made from a blend of flours were found to have 6.87 g, 5.98 g and 41.898 g protein, moisture and fat content respectively. The diameter, height and spread ratio of the protein enriched cookies were 5.5 cm, 0.441 cm and 12.47 respectively. Sensory evaluation was carried out by 10 semi trained panelists for aroma, crispiness, mouth feel, taste, color, flavor, texture and overall acceptability. The overall acceptability was found to be 8 on a 9 point hedonic scale.

Keywords: Protein enriched cookies, Sensory evaluation, Physico-chemical analysis

INTRODUCTION

Millet is a word which originated from the French word '*mille'*, which means thousand, this is because a handful of the grains makes a thousand. The millet belongs to a group of forage grasses which produces small-sized grains. This group of species are said to have originated from China, Asia and Africa. Millets are the sixth biggest cereal crops after wheat, maize, rice, barely and sorghum. These are superior in nature in terms of nutritional quality, have higher protein, aminoacids, macro and micro nutrient content. Millets also have other properties which are not present in cereals like rice or wheat like antioxidant, antimicrobial, anti-inflammatory, antiviral, anticancer, antiplatelet aggregation and catractogenesis inhibitor activities (Adebiyi *et al.*, 2016).

Millets are also known as nutria-cereals, because of their higher vitamin and amino acid content. According to Leder (2004) it contains 22-28% Albumins and Globulins, 28-32% Glutelein and Glutelein like protein and 22-35% Prolamin. They are also rich sources of phytochemicals, polyphenols, soluble and insoluble dietary fibers and minerals. They contain highly polysaturated fatty acids and also have a low glycemic index (Adebiyi *et al.*, 2016).

Millets require only very less water and other resources to grow. They can be used as a sustenance food for communities which do not have enough nutrition. Although these are rich in nutrition they are not used in food in a wide range (Geetha *et al.*, 2014). As the population is growing in size, they require the nutrition provided by the millets to survive (Pu Huang *et al.*, 2016). The millet is small in size and hence, there are high chances of small pebbles mixing in that and making it a bit difficult to eat. They undergo blanching, malting, dry heating, acid treatment, popping, etc., to reduce level of antinutrients, increase shelflife and improve digestibility (Kavitha Patil *et al.*, 2014).

Cookie is a baked product which is available in various shapes and is made mainly using flour, sugar and fat. When

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whole wheat is milled, only the carbohydrate rich endosperm is retained. This results in a big loss of many nutritionally valuable biochemical compounds such as dietary fibre, vitamins, minerals and antioxidant which play an important role in reducing the cardiovascular diseases. The loss of vitamins and minerals in refined wheat flour has led to widespread prevalence of constipation and other digestive disturbances and nutritional disorders (Heshe *et al.*, 2015).

Cookies are generally formulated using refined wheat flour, which doesn't provide much nutrient value. To make it nutritious, a substituted formula of millets and pulses are used in the present research which increases the protein content of the cookies. Soy, bajra and kodo millet flour are incorporated in the making of cookies, which makes it nutritious.

MATERIALS AND METHODS

Materials

The ingredients for the cookies such as millets, whole wheat flour, baking powder, butter, were purchased from a local market in Chennai. The millet was ground in a grinder-mixture to obtain a fine powder, so that there will not be small kodo millet pieces in the cookies.

Preparation of the Cookies

The experimental cookies were made using standard cookie recipe, where a portion of refined wheat flour was replaced by mixture of soy, bajra, kodo millet flour. The butter and sugar were creamed in a hand beater for 3 minutes and the mixture of flours along with baking powder was sifted and added to the creamed mixture. The dry ingredients were then folded into the butter-sugar mixture. Essence was then added to the dough. The dough was then made into shapes and transferred to a slightly greased baking tray. The cookies were then baked in a preheated oven for 10 minutes at 180 °C. The cookies were then removed and allowed to cool for 1 hour before analysis was done.

Table 1: Formulation of Experimental Cookies

Ingredients	Quantity
Refined wheat flour	50 g
Bajra flour	25 g
Kodo millet flour	25 g
Soy flour	45 g
Butter	120 g
Sugar	100 g
Baking powder	5 g
Water	1 tbsp
Vanilla essence	Few drops

Proximate Composition Analysis

Moisture: The moisture for the cookies was analysed by the AOAC method. 5 g of the sample was transferred to the dried and weighed dishes. The sample was placed in the drying oven and dried for 3 hrs at 105 °C, and then cooled in desiccators to room temperature and reweighed (Heshe *et al.*, 2015).

Fat: The fat was determined using a Soxhlet extractor using diethyl ether (boiling point, 55 °C) to extract the fat from a 5 g sample. The ether was evaporated from the extraction flask. The amount of fat was calculated from the difference in weight of the flask before and after extraction as percentage (Heshe *et al.*, 2015).

Protein: The protein is found by Khjeldahl method with the help of concentrated sulphuric acid, copper sulphate and potassium sulphate, these help in the conversion of nitrogen to ammonia. The ammonia which is released after alkalization (using Sodium Hydroxide) is steam distilled into boric acid and titrated with hydrochloric acid (Heshe *et al.*, 2015).

Physical Characteristics

Diameter and Thickness: The diameter and the thickness of the cookies were measured using a Vernier calliper before and after the baking. To measure these 4 samples of the cookies were taken and the total diameter was measured. The cookies were rotated by 90° and the diameter was measured. The average of the two was taken as the final diameter. The height was measured by stacking the 4 cookies and measuring their thickness. The average was taken as the final thickness. The weight of the cookies was measured using an electronic balance (Noor Azhia *et al.*, 2012).

Sensory Evaluation

Sensory evaluation was done for the cookies after the baking and cooling. The cookies were evaluated by 10 semi-trained panellists on the basis of colour, flavour, taste, texture and overall acceptability on a 9 point hedonic scale.

RESULTS AND DISCUSSION

Proximate Composition

The result of the proximate analysis of the experimental cookies made using soy, bajra, kodo millet is shown in the Table 2. The fat content of the cookies was 41.898%, this was required to bind the cookies together. The protein content of the cookies has been enriched due to the addition of soy flour, which is naturally rich in protein.

Cookies diameter, thickness and spread factor is shown in Table 3. The diameter of the cookies has increased from 3.5 cm to 5.5 cm with a slight difference in the height of the cookies. The increase in the diameter is due to the viscosity of the dough. The viscosity of the dough is influenced by the protein content of the dough. The protein forms a web in the dough which will restrict the expansion of the cookie.

Table 2: Proximate Analysis of the Experimental Cookies

Parameter	Values
Moisture	5.98%
Crude fat	41.90%
Crude protein	6.87%
pH	5.5

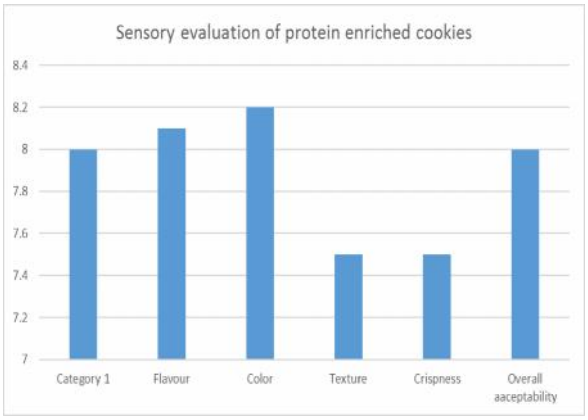
Table 3: Measure of Physical Characteristics of Cookies

Parameter	Before Baking	After Baking
Diameter	3.5 Cm	5.5 Cm
Thickness	0.5 Cm	0.441 Cm

Table 4: Sensory Evaluation of the Experimental Cookies

Sensory Characteristics	Score
Taste	8
Flavour	8.1
Color	8.2
Texture	7.5
Crispness	7.5
Overall acceptability	8

Graph 1: Sensory Evaluation of the Experimental Cookies



Here even though the content of protein is high the expansion of the cookie is high.

Sensory Evaluation

The results of the sensory evaluation is shown in the Table 4. The color of the experimental cookies was darker than any standard butter cookie made with refined wheat flour, due to the addition of soy flour. The experimental cookies made were found to be acceptable to the panellists. The sensory valuation revealed the overall acceptability of the product as 8 on a 9 point hedonic scale.

CONCLUSION

The cookies enriched with soy, bajra and kodo millet was found to have more protein content than the butter cookies. The aim of the study was to increase the level of protein in cookies, this was achieved by the incorporation of soy flour. This study has indicated that acceptable and nutritious cookies can be formulated using a mix of flours of soy, bajra and kodo. The overall acceptability of the cookies was also good.

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And Nutritional Sciences

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Vol. 7, No. 1, January 2018

Research Paper ***Open Access***

HYGIENIC EVALUATION OF COW MILK PRODUCTION IN A RURAL AREA OF NIGER

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Received on: 5th May, 2017 Accepted on: 25th October, 2017

This study aimed to evaluate the hygienic status of cow's milk delivered to a dairy unit located in Say (Niger), representing a typical production area of Sahelian region. Milk from 6 groups of farmers was analyzed throughout a 1-year period (11 sampling sessions). Milk samples were withdrawn both before (at ICP = Intermediate Collection Points) and after the transport (at dairy unit), and analyzed for Total Viable Count, Enterobacteriaceae, Escherichia coli and Coagulase-Positive Staphylococci. High mean values were detected, both at ICP and at dairy unit, for Total Viable Count (6·0±0·7 and 6·9±0·8 log CFU·mL^{-1}, respectively) and Enterobacteriaceae (4.6±1.5 and 4.1±1.3 log CFU·mL^{-1}). A marked variability was observed for *E. coli* and Coagulase-Positive Staphylococci, with a high prevalence of low numbers, but also the presence of highly contaminated samples (>5 log CFU·mL^{-1}). Higher microbial counts were detected during the rainy season. Milk transport influenced negatively the microbial loads, especially when the distance was >10 km. Our results show the need for an improvement of local collection/delivery organization to ensure a hygienic milk supply. With this aim, the frequent training of the farmers and the use of equipped dairy units should be regarded as useful tools.

Keywords: Milk hygiene, Sahelian region, Microbiological contamination, Sampling season, Transport

INTRODUCTION

Milk and dairy products play an important role in the diet of developing countries population, in order to achieve a sufficient energy and protein supply. Their consumption has a growing diffusion in nutritional habits of several African people, and is strongly encouraged by FAO and WHO food security programs. Milk production in Sub-Saharan Africa has to face several obstacles due to climatic conditions (high environmental temperatures and marked rain seasonality) and an insufficient organization of the whole chain (Diop and Abellah, 1996; and Cheng *et al.*, 2017). The marked seasonality of Sahelian climate affects significantly quality and hygiene of milk production, in particular when traditional milking is applied. During the rainy season, from June to September, the presence of mud in the outdoor milking areas could result in high microbial initial contamination of milk, especially due to the use of open recipients, such as calabashes (empty gourds), plastic or metal buckets. During the dry seasons (cool dry season, from October to February, and hot dry season, from March to May), the high quantity of dust can also contaminate significantly the milk; in hot dry season, this factor is

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associated to very high environmental temperatures that allow the rapid growth of spoilage and pathogenic microorganisms. It's known that storage/transport temperatures >20 °C promote the development of lactic acid bacteria causing the milk acidification (pH≤6.5) (Pistocchini *et al.*, 2009) and the proliferation of pathogens such as *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* and *Clostridium* spp. (Mellenberger and Kirk, 2017). In such conditions, the duration of transport represents a critical factor, and international guidelines indicate that milking-refrigeration interval should be limited to 2-3 hours (IDF, 1990; and EU, 1992).

The development of a proper local milk chain is also hindered by wide dispersion of the production, traditional farming and milking, and lack of infrastructures allowing a proper management of the product (efficient roads, hygienic production units, energy supply for refrigerated storage). In Sub-Saharan Africa, during the last decades, several milk collection units have been realized; these structures pretend to give a better organization to milk chain by assuring a reliable collection point to farmers, the possibility of milk refrigeration and quality/hygiene controls (Nkya *et al.*, 2007; Pistocchini *et al.*, 2009; and Belli *et al.*, 2013).

Our study was a part of a research and cooperation project aimed to organize farmers of the Department of Say (Niger) to improve local cow's milk hygiene. A total of 129 local farmers from 19 villages were grouped in the cooperative *Association des producteurs laitiers de Say* (Say cow's milk producers association) - *Hawrinde Biradam* (*APL/Say*), and a small dairy processing unit, with a laboratory for basic microbiological analyses, was built in 2009. This experimental study aimed to evaluate the hygienic status of cow's milk delivered to the dairy unit of Say; in particular, the attention was focused on the trend of bacteriological contamination level during the different seasons and on the influence of transport phase on milk quality.

MATERIALS AND METHODS

Milking, Transport and Collection Procedures

Milking was done twice a day, following traditional procedures in outdoor conditions; each farmer collected the milk in a calabash, and shortly afterwards put it into a recipient (e.g., a bottle). In each village, all the milk was gathered into a single food-contact plastic churn at an Intermediate Collection Point (ICP), then transported by bicycle to the dairy unit by 10 a.m. The churns, supplied by the project, were used to avoid the traditional use of recipients intended for different materials (e.g., fuel), and were washed by a NaOH solution and disinfected by NaClO at the dairy unit after each milk transport.

Milk quantity from each village was very variable, depending on the season and consequently on the availability of feed and water. During the Rainy Season (RS) and the Cool Dry Season (CDS) the good quality of pastures allowed to obtain relatively high production levels (15-20 L/village/day), while during Hot Dry Season (HDS) they fell to about 10 L/village/day.

At the arrival at the dairy unit, milk was evaluated for pH and density. If defined ranges were respected (pH≥6.45, density≥1,028 $g \cdot L^{-1}$), the milk was accepted, filtered and stocked into a cooler tank at 0-1 °C for subsequent sale.

Milk Sampling

For the evaluation of raw milk quality, 6 groups of farmers were selected, involving a total of 21 familiar groups. The groups were chosen considering the distance from the ICPs to the dairy unit (≤10 km for groups 1, 2 and 3; >10 km for groups 4, 5 and 6), to be representative of the whole productive area of Say. A total of 11 sampling sessions were performed during the period August 2011-July 2012, aiming to obtain samples during all the seasons (5 sessions during both RS and CDS, and 1 session during HDS).

Each time, milk samples were withdrawn both at ICP (from the plastic churn before bike transport) and at the dairy unit (at the reception of the churns). Milk samplings were performed by sterile syringe; at the same time, the environmental temperature and the hour of sampling were registered, and milk temperature and pH were measured, using digital pH meter/thermometer (HI 8424, Hanna Instruments, Baranzate, I). The samples withdrawn at ICP were kept refrigerated and transferred to the laboratory within 1 hour, while the samples picked at the dairy unit were analysed immediately.

Microbiological Analyses

Milk samples were put into sterile tubes, and decimal dilution were prepared by NaCl/tryptone saline (0.85%); then, microbiological analyses were performed using a spread plate technique, considering the main bacteriological indicators of contamination. Total aerobic Viable Count (TVC) was performed on Milk Count Agar (MCA,

Biogenetics, Ponte S. Nicolò, I). Plates were incubated at 37 °C for 48 hours. The temperature of 37 °C for the incubation of MCA, instead of the usual value (30 °C) was chosen as it was more representative of the environmental temperatures of the area considered. The number of *Enterobacteriaceae* was determined according to the ISO: 21528-2:2004 method (ISO, 2004); *E. coli* counts were determined according to the ISO: 16649-2:2001 method (ISO, 2001), and Coagulase-Positive Staphylococci (CPS) were enumerated following the ISO: 6888-1:1999 method (ISO, 1999).

Statistical Analysis

All microbiological data were submitted to analysis of variance (ANOVA) using the NLMIXED procedure by SAS/stat package version 8.0 (SAS Inst. Inc., Cary, NC). The sampling point (ICP or dairy unit), the distance of the ICP from the dairy unit ($\leq$10 km or >10 km) and the sampling season were considered as fixed effects. A value of $P<0.05$ was considered statistically significant.

Data concerning milk transport duration, environmental temperature at ICP, and milk temperature and pH both at ICP and at the dairy unit were also submitted to ANOVA, considering the sampling season and the distance as fixed effects. Correlation coefficient (r) of all bacterial counts with values of the parameters registered during sampling was also calculated.

RESULTS AND DISCUSSION

Milk Temperature and pH

Milk temperatures and pH values, and environmental temperatures are reported in Table 1. Milk temperature decreased gradually during the transport, due to the lower environmental temperature (as the delivery was performed within 10 a.m.) and to the application of wet cloths on the churns, as suggested to farmers during the training courses. No statistically significant differences among the data collected in the three seasons were detected. Nevertheless, during the hot dry season, higher milk temperatures both at ICP and at the dairy unit were recorded, with a very low decrease between the two points. A statistically significant correlation was observed between environmental and milk temperatures ($P<0.01$). Milk pH values were within the normal range for similar contexts (Millogo *et al.*, 2010), and didn't show any difference among the seasons.

Microbiological Quality of Milk

The results obtained by microbiological analyses showed high bacterial numbers in milk samples, considering international dairy quality standards, but they were in line with data obtained by several studies conducted in similar climatic and management contexts.

Table 1: Milk Temperatures and pH Values in Relation to the Sampling Season

Parameter	RS	CDS	HDS
Environmental temperature (°C) at ICP	26.8±2.5	28.0±2.0	32.5±2.1
Milk temperature (°C) at ICP	31.7±2.5	33.0±2.9	33.5±2.8
Milk temperature (°C) at dairy unit	30.2±3.1	30.1±4.0	33.1±2.2
pH at dairy unit	6.54±0.06	6.55±0.07	6.55±0.02

Note: ICP = Intermediate Collection Point, RS = Rainy Season, CDS = Cold Dry Season, HDS = Hot Dry Season.

TVC ranged frequently between 6 and 7 log CFU·mL^{-1}; the mean values measured in samples collected at the ICP and at the dairy unit were 6.0±0.7 log CFU·mL^{-1} and 6.9±0.8 log CFU·mL^{-1}, respectively; the difference observed was statistically significant ($P<0.05$). A high frequency of relatively low counts was detected in ICP samples, as more than 50% values were <6 log CFU·mL^{-1} (12% of the dairy unit samples were below this limit), while very high counts (>8 log CFU·mL^{-1}) were observed only in samples withdrawn at the dairy unit (12%). *Enterobacteriaceae* numbers ranged mostly between 4 and 6 log CFU·mL^{-1}, with high variability. Mean values recorded for dairy unit samples (4.6±1.5 log CFU·mL^{-1}) were higher, also if not significantly different ($P = 0.37$) from those detected in ICP samples (4.1±1.3 log CFU·mL^{-1}). About 88% of the samples withdrawn at ICP had counts below 5 log CFU·mL^{-1}, while this rate decreased to 53% in samples from the dairy unit.

Escherichia coli counts were very variable, with a high prevalence of low numbers (<2 log CFU·mL^{-1}) especially in samples withdrawn at ICP (50%), but with high contamination levels (>5 log CFU·mL^{-1}) in some cases; no significant differences were observed between ICP and dairy unit samples ($P = 0.96$). A marked variability was observed also for CPS, with potentially dangerous contamination levels (>4 log CFU·mL^{-1}) in a significant percentage of samples (11 and 36%, at ICP and at the dairy unit, respectively); the comparison of values obtained from ICP and dairy unit samples didn't show any statistical difference ($P = 0.59$).

High contamination values can be linked to traditional milking procedures, such as manual milking in non-dedicated, uncovered areas. The studies conducted in Mali and Burkina Faso, with similar contexts to the area considered by our evaluation, gave comparable counts (Bonfoh *et al.*, 2003; and Millogo *et al.*, 2010), but these values agree also with the results of previous studies carried out in Uganda, Tanzania and Morocco (Lues *et al.*, 2003; Kivaria *et al.*, 2006; Sraïri *et al.*, 2006; Donkor *et al.*, 2007; and Grimaud *et al.*, 2009). The importance of the application of hygienic procedures along the whole production-sale milk chain has been already highlighted by Kouamé-Sina *et al.* (2012), showing the potential reduction of pathogens prevalence and of the number of human foodborne illness cases due to the application of proper practices.

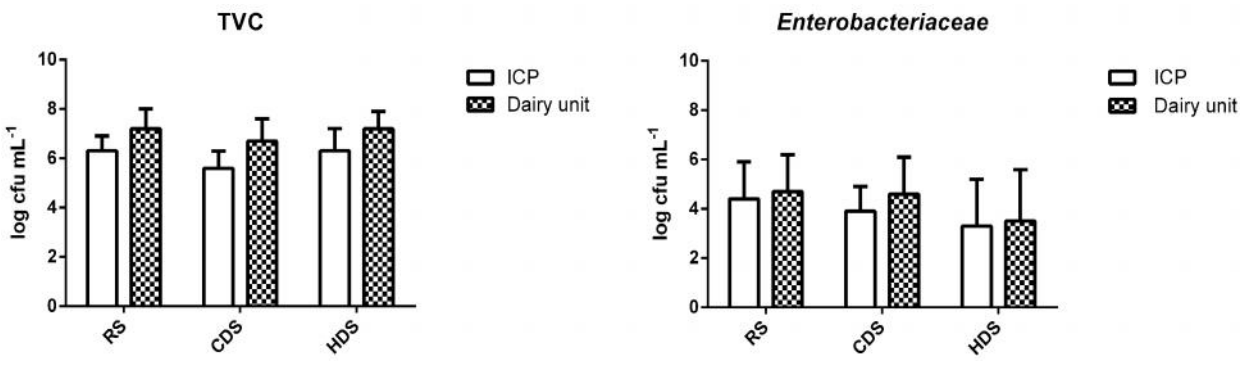
Comparison Among the Sampling Seasons

Data from microbial counts were clustered, based on sampling season, in order to evaluate the influence of climatic factors (temperature, rainfall) on milking hygiene and milk transport. Figure 1 shows the mean values observed for TVC and *Enterobacteriaceae*, while in Figure 2 is reported the frequency distribution analysis of all the data obtained during RS and CDS sampling; due to a too low number of data, HDS values weren't submitted to this analysis.

TVC values were not significantly influenced by the sampling season (P = 0.97), but lower counts were observed in CDS milk samples, and more than 90% of the samples withdrawn at ICP showed values <6 log CFU·mL^{-1}. During RS, higher counts were registered, with 20% of the samples overcoming 7 log CFU·mL^{-1}; this could be due to milk contamination during the rainfall (presence of water and mud on the skin and udder surface). Our data agree with those reported by Sraïri *et al.* (2009), who underlined the clear effect of seasonal climatic variations on the cow's dirtiness, resulting in higher counts during the rainy periods; during HDS, high TVC values could be favoured by the presence of heavy dust diffusion associated with high environmental temperature. A similar situation was observed also in milk samples from the dairy unit; a higher percentage of TVC values >8 log CFU·mL^{-1} was in fact detected in RS and HDS samples.

Also for *Enterobacteriaceae*, no statistically significant influences by the sampling season were observed (P = 0.25), also if slightly higher mean counts were detected during RS if compared to CDS. 20% of samples taken at ICP during RS had counts >6 log CFU·mL^{-1}, while all the counts at CDS were below this limit; for samples withdrawn at the dairy unit, this percentage raised to 25% and 12.5% for RS and CDS, respectively. These results can be explained by the negative influence of rainfall, as for TVC, but the environmental temperature, in this case, didn't seem to have an evident influence on microbial counts. In fact, data concerning HDS samples indicated the presence of lower counts both at ICP and at the dairy unit; such results could be due to the lower possibility of faecal contamination in very dry milking areas, but the small number of data didn't allow a complete analysis. *E. coli* counts weren't significantly different among the seasons (P = 0.13), also if slightly higher values were detected during RS, and frequency distribution of data was similar between RS and CDS. The small number of HDS data confirmed the trend

Figure 1: Total Viable Counts (TVC) and Enterobacteriaceae Counts in Milk Samples Collected in Different Seasons



Note: ICP = Intermediate Collection Point; RS = Rainy Season; CDS = Cold Dry Season; HDS = Hot Dry Season.