Seasonal Effect on Bovine Raw Milk Composition of Oulmes Local Race in Morocco

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Abstract
A total of 120 samples were collected and examined in three seasons: autumn 2013, winter and spring 2014. The milk samples were obtained from Oulmes bovine race located in the Middle Atlas in Morocco. This search work aimed to characterize the physicochemical quality of this milk related to the different seasonal variations.
The physical parameters tested were, moisture, total solids, conductivity and freezing point. Also, some chemical components, including fat, protein, lactose, ash, density and the minerals (Ca, Mg, Na, K, P, Fe, Cu, Mn and Zn) were carried out. Protein, Ca and K levels demonstrated seasonal trends with a maximum of 3.66 ± 0.01%, 1476 ± 167%, and 1173 ± 179% respectively, in spring and conductivity of 4.35 ± 1.45mS in autumn, while minerals and many physical properties displayed considerable variations. Many significant interrelation ships in physicochemical properties were found mainly for protein which was strongly correlated to solid non fat (SNF) (r= 0.94), salts (r =0.75), density (r =0.80), lactose (r = 0.76), and freezing points (r =-0.69).
Keywords: bovine milk, Oulmes race, physicochemical composition, Pearson's correlation.

1. Background

The dairy sector is among the agricultural sector which showed a steady improvement in its production and performances. According to the Moroccan Ministry of Agriculture and Marine Fisheries, milk production continued its upward trend, reaching 2.5 billion liters in 2012 against 1.8 billion in 2008, thus more than 40% of increase in four years [1].

Nevertheless, in early 1970s, Moroccan government launched the dairy plan in order to improve milk production. It concerned a massive importation of heifers from European countries. As consequence of uncontrolled random mating, local breeds have undergone considerable genetic erosion. Its proportion, which was 95% in 1970, decreased to 50% in 2006 [2]. Among the main Moroccan local breeds, Oulmes-Zaer has been identified as early as 1912 and recognized by ministerial decree in 1982. It is an original race, particularly located in the Middle Atlas of Morocco, reared for two purposes, milk and meat productions and well adapted to harsh environment[2]. It is well known that this region is rich in mineral water, and natural mixed vegetation. Furthermore, this biotope is considered to be a natural reserve of aromatic and medicinal plants, such as *Mentha pulegium, Lavendula stoechas, Lavendula angustifolia, Mentha suaveolens, Nerium oleander, Daphne gnidium, Myrthus cummunis*, etc.[3, 4], which may be the feature point of said milk.

It is well known that a variation in characteristics and chemical composition of milk is due to several factors [5, 6]; whether these factors are related to the animal or to intermediates and livestock. Also the seasonal variation has a greater effect on milk composition [7]. In this regard, the aim of this study is to evaluate the physic-chemical composition of bovine milk collected from Oulmes race in Oulmes region, because it's essential for nutritional and technological value [8], and understand the climate and biotope effect on its particular quality.

2. Methods

2.1 Study sites

This study was conducted in rural region called Oulmes, located at150 km at the North-West of Rabat city. This region is set on high mountains of the Middle Atlas and middle hill located to the west, in Berber Tamazight area. It is a mountainous zone provided highlands, located at 1260m above sea level, 33 ° 25 '0 "N and 6 ° 1' 0" W. This region is known by local cattle breed called blonde Oulmes-Zaer, which is traditional and original Middle Atlas race, and reared for both milk and meat. Total herd is estimated to 8000 cows in municipalities of Bouquachmir, Ait Ichou and Oulmes[9].

2.2 Sample collection

To perform this part of research, one sample per cow was collected, from 40 Oulmes race cows. Sampling was carried out in three periods during one year. The first period was between early October and late November 2013, the second period between mid-January and late February 2014and the third time between late March and late May

2014, knowing that the land cover changes from one season to another, and thus to monitor the quality of milk in relation to each period.

Samples were collected aseptically from all animals in natural pasture. Raw milk was sampled in morning by hand milking. The milk of each cow was collected directly in sterile bottles without preservative and kept at 4°C. All samples were analyzed immediately on arrival to the laboratory [10].

2.3 Physical and chemical analyses

2.3.1 Lactoscan milk analyzer

Milk composition (fat, solids non-fat (SNF), density, proteins, lactose, salts, freezing point, and conductivity) of samples directly after collecting and processing was determined using auto-analyzer (LACTOSCAN®Milk Analyzer) [11].

2.3.2 Dry matter

The determination of the dry matter content was carried out according to the AOAC method [12], after drying 5g of milk at a temperature of 103 °C for 3 hours, until water evaporation and weight stabilization.

2.3.3 Ash

The determination of the ash content of the milk samples was performed according to the AOAC method [13]: 3 g of the sample was weighed into a crucible dried to a specific weight then placed in the oven at a temperature of 550 °C for 6 hours to destroy any organic material. After they are cooled, the samples were again weighed once and calculated as ash percent.

2.3.4 Mineral composition

In this step of the present search work, mineral elements were monitored according to their specificity. The Na^+ and K^+ were evaluated using flame photometer and Ca^{2+}using complex-metric titration. The phosphorus content was determined by reaction of the acidified solution of ammonium molybdate containing ascorbic acid and antimony [14]. Reading results was performed at 825 nm with UV-visible spectrophotometer (Jenway 6405 UV / VIS spectrometer) [15].

Trace elements, namely iron, copper, manganese, zinc and magnesium were performed using atomic absorption spectrometry in an air-acetylene flame [16]. The measured absorbance was determined at a specific wavelength of 248.3 nm and the data was studied and interpreted.

3. Statistical Analysis

Data was statistically treated using variance analysis (ANOVA), results are shown as mean ± standard deviation, and differences among means were ranked using Duncan's New Multiple Range Test. The significance level was 5%.

4. Results and discussion

4.1 Chemical analysis

Physicochemical results of analyzed milk samples collected during the different seasons are presented in Table 3. Among the main physicochemical properties used in the dairy industry there are the density and freezing point [17]. The results showed that the highest density was 1032 Kg/m^3 in spring and the lowest was 1030 Kg/m^3 in autumn. We note that these values are similar to those reported by Chen *et al.*(1026-1031 Kg/m3)[18].The normal density of cow's milk is around 1030 to 1035 Kg/m3, it varies according to the increase of dry matter and is inversely related to fat content[19].

Moreover, all milk treatment or changes in its composition induce a change in it freezing point, which varies between -0,530 and -0,562 °C in spring. It presents a significant variation between seasons, and remains close to the average found in other study [20].

However, the average fat content, with a range of 2.85 to 3.25%, was consistent to the AFNOR value (3.04%)[21],this value remains similar to that found in Brazil (3.63%) [22].It is also a close value compared to the results found by Mapekula*et al*. in the milk of local Nguni cattle in South Africa (3.25%)[23, 24].

For Dutch raw bovine milk, the maximum was 4.57% in January. This average is lower to 6.09% found in autumn as a maximum value of fat, which could be attributed to different temperatures and food composition [25]. Cows get dry food in winter, and grass in summer as they are outside for long time [26]. Fat variability depends on several factors such as weather conditions, stage of lactation and feeding. Milk protein content is also subject to seasonal changes and locality and their variations are higher, compared to those of the fat content[27].

In this work amount of milk protein of Oulmes breed was found to be in the range of 2.13 to 4.02% with a mean of 3.58%. This value is higher than that found in Holstein cow milk of Lordegan, region of Iran (3.30%)[28], and then that found in Chinese Holstein cows in Northern China(3.10%)[29].Also, the values observed in this research are higher when compared to those found by Imran *et al*. in Pakistan (3.28%), Ponka *et al*. in Cameroon (3.26%), and Cofani *et al.* in Brazil (3.27%)[30, 31, 22].

Table 3.Composition of whole milk obtained during different seasons from Oulmes region.

	Season	Mean ± SD	Min	Max
Density (Kg/m^3)	Autumn	1030 ± 3**b**	1012	1038
	Winter	1031 ± 1**a**	1025	1037
	Spring	1032 ± 2**a**	1027	1037
Freezing point (°C)	Autumn	-0.530 ± 0.067**a**	-0.737	-0.418
	Winter	-0.540 ± 0.059**a**	-0.675	-0.453
	Spring	-0.562 ± 0.066**b**	-0.686	-0.014
Conductivity (mS)	Autumn	4.35 ± 1.45**a**	1.60	8.25
	Winter	3.80 ± 0.96**b**	2.40	7.52
	Spring	3.06 ± 0.88**c**	0.90	4.77
SNF (%)	Autumn	5.85 ± 0.04**a**	3.52	6.74
	Winter	5.93 ± 0.02**a**	5.41	6.39
	Spring	6.07 ± 0.02**b**	5.47	6.55
Salt (%)	Autumn	1.65 ± 0.01**a**	0.93	1.91
	Winter	1.67 ± 0.01**ab**	1.53	1.97
	Spring	1.69 ± 0.01**b**	0.56	1.91
Fat (%)	Autumn	3.10 ± 0.14**a**	0.74	6.09
	Winter	3.02 ± 0.14**a**	0.66	6.08
	Spring	3.01 ± 0.12**a**	0.66	5.10
Protein (%)	Autumn	3.51 ± 0.02**a**	2.13	4.02
	Winter	3.57 ± 0.01**b**	3.24	3.97
	Spring	3.66 ± 0.01**c**	3.29	4.01
Lactose (%)	Autumn	4.31 ± 0.03**a**	2.60	4.95
	Winter	4.37 ± 0.01**ab**	3.98	4.94
	Spring	4.44 ± 0.02**b**	1.80	4.86
Dry matter (%)	Autumn	70.21 ± 0.05**a**	58.71	81.15
	Winter	71.03 ± 0.05**a**	56.85	83.43
	Spring	71.37 ± 0.04**a**	60.68	82.51
Ash (%)	Autumn	0.54 ± 0.02**a**	0.49	0.61
	Winter	0.51 ± 0.03**b**	0.44	0.59
	Spring	0.50 ± 0.02**b**	0.45	0.57

^{abc} Different letters for the seasons in the same parameter indicate significant difference (P<0.05).
SNF = solids-not-fat

Generally, protein content of milk is an essential feature of its market value since higher protein content enhances performance of technological transformation; Milk with high protein content is good for cheese production [9]. These Oulmes milk samples be havior give a good indication that this raw milk can be considered as economical material for the dairy industry in this special zone.

According to Pougheon and Goursaud[32], season has an important influence. Protein was constantly lower during autumn compared to spring and winter; this result is in agreement with that reported by Heck *et al.* [25]. This was probably due to lower intake of feed energy with in-door feeding, and may reflect the particular feeding regime used for this herd.

Protein is the major structural component of all cells in the body and functions in the form of enzymes, transport carriers, and hormones. Proteins are rich sources of peptides that significantly lower blood pressure in those with hypertension and may contribute to satiety and regulate food intake [33].

Milk contains carbohydrates, mainly lactose which is the main sugar of milk, which is involved in the intestinal absorption of calcium, magnesium and phosphorus, and the utilization of vitamin D; lactose also provides a ready source of energy, and constitutes a substrate for lactic acid bacteria [34]. Mathieu reported a value of 4.37 %[20], this value was close to that found by SanzCeballos *et al.* in Holstein Friesian cow milk (4.47 %), Cofani *et al.* in Brazil (4.43 %),and Imran *et al.* in Pakistan (4.38 %)[23, 22, 33].Lactose is one of the least variable milk components. Our results showed a small variation of lactose, ranging from 4.31 % in autumn to 4.44 % in spring. The same conclusion was reached by Heck *et al.* for Dutch bovine milk [25].Thus lactose is the least sensitive component of milk to dietary changes as concluded by Walstra *et al.*[35].

Considering ash content, a value of 0.50% in spring and 0.54% in autumn was found, with a maximum of 0.61%. These contents are close to 0.63% in Cameroon[31], and to 0.65 % in Holstein Friesian cow milk of South Eastern Spain[23], but are lower than those for raw milk of Malian zebu cows (0.79%)[36].Effect of season

on ash content was small and this is in concordance with results reported previously [37].
For dry matter content, we found that there is no significant seasonal effect on raw milk. Average value was about 71.03 %, with a maximum of 83.43 %. This value was close to that reported by Imran *et al.* in Pakistan (86.8%)[30].

The conductivity range of the milk samples was shown from 3.06 to 4.35 mS, close to that found by Parasca and Chilimar (3.67 mS)[38].The maximum was 8.25 mS observed for autumn milk samples, which is close to 9.20 mS found in Pakistan [30].Milk has conductive properties because of the existence of charged compounds such as salts[26]; nevertheless, Mabrook and Petty suggest that the physical and chemical nature of the casein micelles can influence the overall milk conductivity[39].

4.2 Mineral analysis

Changes in levels of trace elements in bovine milk depend on nutrition, race, age, stage of lactation, location, production rates, as well as analysis method. The result of the mineral content of the statistical analysis, in the different milk samples in the three seasons, using one way ANOVA at 95% confidence level are shown in Table 4.

Table 4. Mineral composition of raw cow milk collected in Oulmes region in different seasons.

Element (ppm)	**Season**	**Mean ± SD**	**Min**	**Max**
Ca	Autumn	1246 ± 171**c**	1030	1703
	Winter	1349 ± 170**b**	1000	1656
	Spring	1476 ± 167**a**	1151	1887
Mg	Autumn	148.02 ± 26.86**a**	110.15	224.94
	Winter	156.72 ± 28.30**b**	104.70	266.62
	Spring	159.51 ± 32.56**b**	100.02	208.98
Na	Autumn	400.49 ± 82.81**b**	265.34	687.91
	Winter	477.43 ± 66.01**a**	331.02	579.53
	Spring	413.03 ± 52.09**b**	307.56	545.90
K	Autumn	1047 ± 221**b**	557	1860
	Winter	877± 109**c**	648	1158
	Spring	1173 ± 179**a**	870	1609
P	Autumn	970± 31**b**	832	1036
	Winter	1068 ± 224**a**	509	1760
	Spring	1004 ± 160**b**	705	1467
Fe	Autumn	0.288 ± 0.15**b**	0.10	0.75
	Winter	0.586 ± 0.84**a**	0.01	3.50
	Spring	0.190 ± 0.09**b**	0.10	0.55
Cu	Autumn	0.034 ± 0.01**a**	0.02	0.09
	Winter	0.029 ± 0.01**b**	0.02	0.05
	Spring	0.029 ± 0.01**b**	0.01	0.11
Mn	Autumn	0.077 ± 0.03**b**	0.01	0.17
	Winter	0.139 ± 0.31**b**	0.01	1.47
	Spring	0.602 ± 0.54**a**	0.06	1.88
Zn	Autumn	3.095 ± 1.34**a**	1.02	6.70
	Winter	2.165 ± 1.02**b**	1.00	5.50
	Spring	3.019 ± 0.84**a**	1.80	5.40

[abc]Different letters for the seasons in the same parameterindicatesignificant difference(P<0.05).

According to the medicinal aspect, it is known that calcium is responsible for many regulatory functions such as normal cardiac rhythm maintenance, blood clotting, hormone secretion, muscle contraction and enzyme activation. Magnesium also plays an important role in many physiological processes such as metabolism of proteins and nucleic acids, neuromuscular transmission and muscle contraction, bone growth and blood pressure regulation. Magnesium is also a co-factor of many enzymes [40, 41].

Average calcium and magnesium concentrations followed a similar trend. Its minimum was observed during autumn while the maximum was for spring milk samples, with values of 1246 and 1476 ppm for calcium, and 148.02 and 159.51 ppm for magnesium, respectively. These concentrations were higher than those found in Tunisia, Cameroon and in Holstein Friesian cows of South Eastern Spain which were 1000, 1217, and 1135 ppm for Ca and 70, 103 and 94 ppm for Mg, respectively [42, 31, 23].

Sodium to potassium ratio is necessary to human health. It maintains blood plasma equilibrium with milk, which is isosmotic, there will be a variation in the concentration of Na, which is offset by variation of K, Na/K ratio can then be used to assess the health of dairy cattle. For the Na and K content, the average concentrations ranged between 400.49 and 477.43ppm, and 877.69 and 1173 ppm, respectively. Values of 310 and 1384 ppm were reported by Ponka*et al.* in Cameroon, and of 430 and 1500 ppm by Sboui*et al.* for South Tunisian cows [31, 42].

It is indicated that phosphorus is a major element with many important biological functions in the human body. The present work showed that its content varies according to the seasons. It reaches a maximum in winter with an average of 1068 ppm and a minimum of 970.43 ppm in autumn. These are slightly higher when compared to those founded by Ponka *et al*. and Sanz Ceballos *et al*., who reported 776 and 870ppm, respectively [30, 23].

It is well known that Zn is essential for physiological processes, lipid metabolism, brain and immune function. A Zn deficiency causes a body's sensitivity to various diseases and viral, bacterial and fungal infections. However, iron as an essential trace element and participates as a catalyst in several metabolic reactions [43]. Cu is an essential element in nutrition and is very important for human health, as co-factor of enzymes in glucose metabolism and synthesis of hemoglobin, connective tissues and phospholipids [44]. In this study, average values of trace elements varied between 0.190 and 0.586, 0.029 and 0.034, 0.077 and 0.602, and 2.165 and 3.095 ppm for Fe, Cu, Mn and Zn, respectively. The levels of trace elements were within some published ranges, and higher in other studies. Concentrations reported in the literature for cow's milk are0.3, 2, 0.1 and 0.02 ppmfor Fe, Zn, Cu and Mn, respectively [45].

4.3 Interrelationships between physical and chemical parameters

Significant correlations between the main milk components are shown in table 5. Positive significant correlations ($P < 0.05$) between milk concentrations of fat, protein, solids-non-fat, density, salts, lactose and freezing point, in the three seasons were detected for these elements. Protein was strongly correlated to solid not fat (SNF)($r= 0.94$), salts($r =0.75$), density ($r =0.80$), lactose ($r = 0.76$), and freezing points ($r =-0.69$). However its correlation was weak with fat ($r =0.43$). Correlation of fat was also weak to all components, especially to density which is absent ($r = 0.05$). Yang and al. made the same observation for Chinese Holstein cow milk [29]; while for all the other components, the correlation was positively significant and important, and this was in agreement with the results therefore obtained.

It is clear that the Oulmes bovine milk would be more suited to the manufacture of different products at different times of the year, in this way it is hoped to provide some clearer guidelines on the relationships in milk composition, properties and product manufacture, including seasonal factors.

Table 5.Correlation coefficients between raw milk compositional parameters over the three seasons

	Fat	**Protein**	**SNF**	**Density**	**Salts**	**Lactose**	**Freezing Point**
Fat	1	0.43	0.45	0.05*	0.44	0.37	-0.61
Protein		1	0.94	0.80	0.75	0.76	-0.69
SNF			1	0.82	0.74	0.77	-0.71
Density				1	0.57	0.63	-0.47
Salts					1	0.77	-0.59
Lactose						1	-0.61
Freezing Point							1

All the correlation coefficients are significant at the 0.0001 level except this * is not significant.

5. Conclusion

The values of the majority of components for the milk samples analyzed and collected from Oulmes race bovine milk, such as protein, lactose and mineral elements were important. Seasonal variations were found for some components and reviews some of them have been highlighted. On the other hand, analysis of photochemical and fatty acid profile constitute our future research objective in order to proceed to the promotion and production of healthy food made from raw milk Oulmes cattle which can unequivocally contribute to socio-economic and sustainable development of this region.

6. References

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