Abstract:
Milk technological and physicochemical characteristics are vital to cheese yield and quality, but can vary in response to factors such as season. An evaluation was done of milk technological and physicochemical characteristics in the dry and rainy seasons, and their effects on the physicochemical characteristics of traditional Oaxaca cheese. Milk and cheese samples were collected from 21 different small-scale processing plants. Milk samples were analyzed for fat and protein content, acidity level, coagulation time, curd firmness and yield. Cheese samples were analyzed for fat and protein content, acidity level, moisture content and chlorides level. A one-way ANOVA was used to evaluate inter-seasonal changes. Differences by season were observed in milk fat and protein contents, acidity level, coagulation time, curd firmness and yield. In the cheese inter-seasonal differences were present in fat content, acidity level and moisture content. Season clearly affected milk physicochemical and technological characteristics, and consequently cheese composition.

Keywords: Cheese, Milk, Traditional, Technological properties, Physicochemical properties.
Introduction

Various factors effect raw milk composition, including cow breed, feeding regimen, season, and lactation stage\(^{(1,2,3)}\). Differences in these and other factors are vitally important in the fabrication of dairy products such as cheeses because they can influence milk technological properties which are reflected in cheese yield and quality\(^{(4,5)}\), coagulation time and gel firmness.

Higher fat and protein concentrations together with favorable technological parameters can shorten coagulation time and produce greater curd firmness\(^{(4)}\). This means there is a positive correlation between milk fat and protein percentages and cheese yield; in other words, higher solids concentrations result in higher yields\(^{(6)}\). However, cheese quality and performance is not only due to solids volume, but also to protein quantity and quality\(^{(7)}\). Because of its influence on technological parameters\(^{(8)}\), protein is a crucial component in cheeses. This is mainly in response to k-casein\(^{(9,10)}\), a phenomenon confirmed in reports of good coagulation in milk containing high k-casein levels, as well as adequate calcium and pH levels\(^{(11,12)}\).

Seasonal variations can strongly influence milk physicochemical properties and therefore cheese quality\(^{(13,14)}\). This is due to variation in milk fat, protein and lactose percentages during an annual cycle; these are normally lower in spring-summer and higher in autumn-winter. Variation in these percentages responds mainly to changes in cow diet\(^{(15)}\).

Research on seasonal variations in milk has been done worldwide, but is scarce for milk from certain regions of Mexico and has not been linked to cheese quality.

In central Mexico, Oaxaca-style cheese is handmade using artisanal techniques. One of the most widely known cheese types in the country, this traditional fresh cheese product belongs to the pasta filata (or stretched-curd) group of cheeses. It is made by kneading curdled raw milk in hot water until it becomes flexible and forms bands which are then wound into skeins\(^{(16)}\). Because raw cow’s milk is used this cheese type is influenced by any variability in milk properties originating in the animal or external factors\(^{(17,18,19)}\). Raw milk composition and technological properties can also affect dairy product nutritional value\(^{(15)}\), especially in cheeses\(^{(20)}\).

Research has been done on the elaboration and physicochemical characteristics of Oaxaca cheese\(^{(21)}\), and on the relationship between cheese flavor and texture\(^{(22)}\). However, information is needed on how seasonal variation in milk quality affects Oaxaca cheese quality since fat content and density is known to vary widely in the milk produced by the
small-scale milk production systems that supply artisanal Oaxaca cheese makers\(^{(23)}\). Since this cheese is made year round it is important to understand if variations in milk quality affect cheese yield and quality. The present study objectives were to document any seasonal variations in the technological and physicochemical properties of the raw milk used to make traditional Oaxaca-style cheese and if these correlate to cheese physicochemical characteristics.

**Materials and methods**

**Milk and cheese samples**

Three sets of milk and cheese samples were collected directly from small-scale dairies in the northwest of the state of Mexico, Mexico\(^{(23)}\). Samples were collected during the dry season (February-April) and rainy season (August to October). All samples were stored at 4 °C while transported to the laboratory and analyzed within 24 h.

**Milk analysis**

*Physicochemical Parameters:* Fat (F) and protein (P) contents were analyzed using ultrasound waves in an Ecomilk Analyzer KAM98-2\(^{nd}\)\(^{(21)}\), and acidity measured using 0.1 M NAOH/phenolphthalein as an indicator.

*Technological parameters:* Coagulation time (CT), curd firmness (CF) and yield (YD) were measured. For each test the samples were heated to 35 °C, 10% industrial rennet immediately added, and temperature kept constant in a water bath. Measurement of CT was done by monitoring coagulation onset based on a sudden increase in viscosity\(^{(24)}\) as measured with a viscometer (Model LVT, Brookfield Engineering Labs, Inc., Middleboro, MA 02346), using a No. 1 spindle at 12 rpm. Curd firmness (CF) was measured 30 min after addition of the rennet, and calculated as compression force (N)\(^{(25)}\) using a texture analyzer (TX-XT2) with a 10 mm diameter cylindrical probe, 20 mm depth compression cycle and 1 mm/sec velocity. Yield (YD) was quantified by centrifuging to
force serum release. First, 1 ml sample (milk at 35 °C with 10% rennet) was added to Eppendorf tubes, allowed to sit for 30 min and centrifuged (Universal 320R, D-7852Tuttlingen Heftich) at 14,000 rpm for 30 min at 35 ºC(26). Cheese yield was calculated by subtracting initial sample weight from that of the curd.

**Cheese analysis**

Acidity was measured using 0.1 M NAOH /phenolphthalein as an indicator(21). Standard AOAC (1990) methods were used to quantify fat (933.05), protein (991.20) and moisture (926.08); NaCl was measured following the Volhard method(27).

**Statistical analysis**

Milk and cheese data were analyzed with a one-way (season) ANOVA, and differences between means calculated with Tukey’s test ($P<0.05$). Relationships between milk physicochemical and technological properties were analyzed with a Pearson’s correlation. All analyses were done with the STATGRAPHICS Plus statistical package.

**Results and discussion**

**Milk physicochemical and technological properties**

Milk acidity, and protein and fat contents differed by season (Table 1), with higher fat and lower protein values in the rainy season ($P<0.05$). Previous studies(23) evaluating milk physicochemical quality found differences only in fat percentage and density, with higher fat values during the rainy months (average 3.62 %); this is similar to the present results.
Studies done from June to October\(^{(21)}\) report 3.34 % fat, and 3.05 % protein, and another reported 3.18 % fat and 2.97 % protein\(^{(28)}\); all these values are well below those observed in the present study.

Changes in milk physicochemical and technological properties in different seasons are caused by forage availability in livestock feed. For instance there are reports of higher fat and protein percentages in the rainy season when cow diets consist mainly of green forages such as clover\(^{(29,30)}\).

Table 1: Analysis of means for milk physicochemical and technological properties in dry and rainy seasons

<table>
<thead>
<tr>
<th></th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Acid (^{(oD)})</th>
<th>CT (minutes)</th>
<th>CF (Newton)</th>
<th>YD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>3.28(^a)</td>
<td>3.02(^a)</td>
<td>23.6(^a)</td>
<td>16.7(^a)</td>
<td>0.11(^a)</td>
<td>12.6(^a)</td>
</tr>
<tr>
<td>Rainy</td>
<td>3.64(^b)</td>
<td>2.94(^b)</td>
<td>23.1(^b)</td>
<td>27.0(^b)</td>
<td>0.09(^b)</td>
<td>10.8(^b)</td>
</tr>
<tr>
<td>SEM</td>
<td>0.014</td>
<td>0.004</td>
<td>0.024</td>
<td>0.565</td>
<td>0.001</td>
<td>0.082</td>
</tr>
</tbody>
</table>

CT = coagulation time; CF = curd firmness; YD = yield; \(^{(oD)}\) = Dornic degrees.
SEM = Standard error of the mean.
\(^{ab}\) Different letter superscripts in the same columns indicate significant difference \((P<0.001)\).

Milk technological properties differed between seasons, with lower CT, and higher CF and YD in the dry season \((P<0.001)\). Fat and protein percentages are linked to desirable technological properties (lower CT and greater CF), as well as higher YD\(^{(23)}\). However other factors such as protein content and quality (k-casein content, micelle characteristics, genetic polymorphism)\(^{(31-34)}\), and calcium content and pH\(^{(11,12)}\), can influence technological properties. Further research is needed to closely analyze these factors.

The correlation between protein and YD, CF and CT was strongest in the dry season, but for fat it was only positive for YD. Other studies report higher yields and shorter coagulation times at high protein and low fat concentrations\(^{(9)}\). In another study shorter coagulation times (14.61 min) and better firmness (62.63 mm) were observed in the winter rather than in the summer (16.84 min and 59.33 mm, respectively), probably due to higher milk fat content in the winter\(^{(35)}\). A study done in a traditional dairy system found 3.41 % protein content, 4.17 % fat content and 6.06 min coagulation times in winter, but 3.48 % protein, 3.93 % fat and 3.26 min coagulation time in the summer\(^{(36)}\).

Some reports establish that at coagulation times longer than 30 min milk is no longer considered fit for cheese production\(^{(37,38)}\). In the present results 14% of the dry season samples and 33% of rainy season samples exhibited coagulation times in excess of 30 min. The presence of green forage in the cow diet reduces milk enzymatic coagulation
time, mainly because of its effect on milk protein (caseins and whey proteins)(36); this is probably why more rainy season samples exceeded the 30 min limit.

Correlation between milk physicochemical and technological properties

Both CF and YD exhibited a negative correlation with CT in the dry (Table 2) and rainy seasons (Table 3). This coincides with a previous study in which gel was found to be weaker at longer coagulation times(26).

In the present results acidity was also negatively correlated with CT. Other studies mention shorter coagulation times as acidity increases, as well as greater curd firmness(23,39). Reports of shorter coagulation times at low pH values illustrate the effect of low pH on coagulation capacity(37).

Table 2: Correlation coefficients between milk physicochemical and technological properties in the dry season

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>CF</th>
<th>YD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>-0.149</td>
<td>0.169</td>
<td>0.388***</td>
</tr>
<tr>
<td>Protein</td>
<td>-0.233**</td>
<td>0.329***</td>
<td>0.286**</td>
</tr>
<tr>
<td>Acidity</td>
<td>-0.534***</td>
<td>0.441***</td>
<td>0.032</td>
</tr>
<tr>
<td>CT</td>
<td>1.000</td>
<td>-0.608***</td>
<td>-0.250**</td>
</tr>
<tr>
<td>CF</td>
<td>-0.608***</td>
<td>1.000</td>
<td>0.104</td>
</tr>
<tr>
<td>YD</td>
<td>-0.250**</td>
<td>0.104</td>
<td>1.000</td>
</tr>
</tbody>
</table>

CT= coagulation time; CF= curd firmness; YD= yield.

**P<0.01; ***P<0.001

The correlations between total solids (fat and protein) and CT, CF and YD varied in both seasons (Tables 2 and 3). Fat content positively correlated with YD in both seasons, but protein positively correlated to YD only in the dry season. This lack of clear correlation is noteworthy since previous studies report clearly improved CF as casein and protein percentages increase(26,38,40).
Table 3: Correlation coefficients between milk physicochemical and technological properties in the rainy season

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>CF</th>
<th>YD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>-0.030</td>
<td>0.067</td>
<td>0.328***</td>
</tr>
<tr>
<td>Protein</td>
<td>-0.203*</td>
<td>-0.069</td>
<td>0.08</td>
</tr>
<tr>
<td>Acidity</td>
<td>-0.660***</td>
<td>0.121</td>
<td>0.039</td>
</tr>
<tr>
<td>CT</td>
<td>1.000</td>
<td>-0.576***</td>
<td>-0.405***</td>
</tr>
<tr>
<td>CF</td>
<td>-0.576***</td>
<td>1.000</td>
<td>0.350***</td>
</tr>
<tr>
<td>YD</td>
<td>-0.405***</td>
<td>0.350***</td>
<td>1.000</td>
</tr>
</tbody>
</table>

CT= coagulation time; CF= curd firmness; YD= yield.
*P<0.05; ***P<0.001.

Correlations have also been reported between milk components (e.g. fat, protein and calcium, among others) and curd firmness and curd bifurcation time, but not with coagulation time\textsuperscript{[41]}. Although the inter-season correlations did not coincide in the present results, they do meet the conditions in previous reports. As mentioned previously, differences between studies can be attributed to variations in feeding conditions.

**Traditional Oaxaca cheese physicochemical properties by season**

Cheese fat content, acidity and moisture were all higher (\(P<0.05\)) in the rainy season (Table 4). These results differ somewhat from a study of Ricotta and Pecorino cheeses in which milk fat and protein percentages did not differ between seasons (spring, summer, fall and winter), but Ricotta cheese fat, protein and moisture contents did differ between seasons (\(P<0.05\))\textsuperscript{[42]}. In the present results only the milk and cheese fat percentages were proportionately related to season, being higher in the rainy season and lower in the dry season. Protein content was not linked to season, even though yield was higher in the dry season (12.6 %). Although the solids ratio in milk and cheese strongly affects final product characteristics, it is also important in yield. Indeed, cheese yield is directly related to milk fat and casein contents\textsuperscript{[43]}. In the traditional Oaxaca cheese evaluated here protein was the parameter that most directly influenced yield.
**Table 4:** Physicochemical characteristics in traditional Oaxaca cheese by season

<table>
<thead>
<tr>
<th></th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Acidity (%)</th>
<th>Moisture (%)</th>
<th>Chlorides (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry season</td>
<td>20.6ª</td>
<td>18.5</td>
<td>0.8ª</td>
<td>50.4ª</td>
<td>4.2</td>
</tr>
<tr>
<td>Rainy season</td>
<td>22.2ª</td>
<td>18.7</td>
<td>1.0ª</td>
<td>51.3ª</td>
<td>4.3</td>
</tr>
<tr>
<td>SEM</td>
<td>0.103</td>
<td>0.078</td>
<td>0.011</td>
<td>0.104</td>
<td>0.067</td>
</tr>
</tbody>
</table>

SEM = Standard error of the mean.

ªª Different letter superscripts in the same column indicate difference (P<0.05).

**Conclusions and implications**

Season exerted a significant effect on the physicochemical and technological characteristics of milk as well as cheese composition. This effect was mainly due to seasonal variation in feed availability. Although fat, protein and acidity percentages positively correlated to milk technological properties, protein content had the greatest effect on yield, which was reflected in cheeses with low fat and moisture contents. Variation in milk and cheese quality between dairies is typical of traditional products produced with non-standardized processes. The present results provide technological guidelines to quantify this variation, and highlight that it is part of the craft cheese process which makes traditional Oaxaca cheese unique.

**Acknowledgements**

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