EFFECT OF BACTERIAL CULTURE, FERMENTATION TIME AND INOCULUM SIZE ON QUALITY OF EMASI PRODUCED IN THE KINGDOM OF ESWATINI.

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Abstract

Emasi is a dairy product produced by fermenting milk at room temperature. Traditionally fermented milk may have low hygienic quality hence it is important to produce emasi that resembles the traditionally produced product with improved hygienic qualities. The aim of the study was to determine the effect of bacterial culture, fermentation time and inoculum size on sensory attributes and physiochemical properties of emasi. Commercial mesophilic lactic acid culture, isolated Leuconostoc mesenteroides ssp. mesenteroides/ dextranicum and Lactococcus lactis ssp. lactis, mixed culture from emasi sorghum meal and mixed culture from traditional emasi were used for fermentation for 12, 18 and 24 hours; at 2.5%, 5% and 10% (v/v) inoculum size. The samples were examined for physiochemical properties and sensory attributes. The effect of type of culture on physiochemical properties was significantly different (P<0.05) between the sources. Titratable acid ranged from 0.96% to 1.22%, whilst viscosity ranged from 3551cP to 2222cP. The isolated bacterial strains had the lowest syneresis percentage whilst mixed culture from emasi sorghum meal had the highest. It ranged from 48.6% to 56.5%. The pH ranged from 4.26 to 3.87. The overall acceptability by the panellists showed that they preferred the commercial mesophilic lactic acid bacteria and isolated bacterial strains compared to the mixed culture of traditional emasi and emasi from sorghum meal produced emasi. Fermentation time and inoculum size did not have any significant effect on the physiochemical properties and sensory attributes of emasi. More studies need to be done on isolating bacterial strains that will produce characteristics that resemble the traditional product emasi.

Keywords: emasi, bacterial culture, inoculum size, fermentation

INTRODUCTION

Milk is a nutritious and essential food for human beings. It also serves as a good medium for the growth of many microorganisms (Robinson and Tamime, 1981, Dlamini et al., 2015). Common micro-organisms that may grow in milk are Lactobacillus, Streptococcus, Staphylococcus and Micrococcus spp. (Srujana et al., 2011). Milk from cows is consumed as fresh milk or fermented milk as part of a balanced diet for Eswatini households. Fermentation is the oldest means of preserving food (Robinson and Tamime, 1981). Traditionally fermented milk products are abundant in sub-Saharan Africa and have been widely reported (Mutukumira et al., 1996; Gadaga et al., 1999).

In Eswatini, fermented foods have been produced since time immemorial and traditionally fermented milk is known as emasi (Masarirambi et al., 2009). Due to the location of Eswatini (31°S), the country enjoys a sub-tropical climate (Thompson, 2003), which is favourable for the production of traditionally fermented products like emasi (Masarirambi et al., 2009). Emasi is a fermented dairy milk product obtained from fermentation with mesophilic lactic-acid-producing microorganisms. It may be drunk as a refreshing nutritional drink or used as a relish with the staple food (lipalishi and
The fresh milk was pasteurised at 85°C for 15 minutes before inoculating with culture. The culture was inoculated at 29°C-32°C and left to ferment at room temperature. After fermentation the emasi was cooled at 4°C for 24 hours before carrying out the tests.

**Preparation of lactic acid culture**

**Commercial lactic acid culture**
The raw milk was sterilized at 121°C for 15 minutes and cooled to 30°C before inoculating. Two granules of the freeze-dried mesophilic aromatic lactic acid culture (CHN-22, CHR-Hansen, South Africa) were added into a 250mL of sterilized milk. The milk was incubated at 30°C for 24 hours and refrigerated at 4°C before using it as a starter culture for research.

**Mixed bacterial culture from emasi sorghum meal**
The raw milk was sterilized in an autoclave at 121°C for 15 minutes and cooled to 30°C before inoculating. Fifteen mL of traditional sorghum emasi was inoculated into 250 mL of sterilized milk. The milk was incubated at 30°C for 24 hours and refrigerated at 4°C before using it as a starter culture for research.

**Mixed bacterial culture from traditional emasi**
Fifteen mL of traditional emasi was inoculated into 250 mL of sterilised milk. The milk was incubated at 30°C for 24 hours and refrigerated at 4°C before using it as a starter culture for research.

**Isolated Leuconostoc ssp. and Lactococcus ssp. culture**
The pure strains of *Leuconostoc mesenteroides* ssp. *mesenteroides*/*dextranicum* and *Lactococcus lactis* ssp. *lactis* were isolated at the department of Environmental Health Sciences, Faculty of Health Sciences, Mbabane Campus, University of Eswatini. Pure strains of *Leuconostoc mesenteroides* ssp. *mesenteroides*/*dextranicum* and *Lactococcus lactis* ssp. *lactis* were obtained separately by growing in MRS broth. The purity of the lactic acid culture was determined by inoculating a loop of each strain on to the MRS agar (Merck Laboratories, Darmstadt, Germany). They were incubated for 48 hrs at 30°C.

Lactic bacteria broth (LBB) was prepared by adding 1% tryptone, 0.5% yeast extract, 0.2% lactose and 1.0% sodium chloride (NaCl) into 200 mL distilled water. The broth was sterilised at 121°C for 15 minutes and cooled to 30°C before inoculating. A colony of the culture strain growing in the petri dishes were inoculated into the broth (30°C) growing separately. They were incubated for 48 hrs at 30°C.

Sterilized skimmed milk was inoculated with 1ml of lactic bacteria broth incubated with pure strains separately. The pure strains were separately grown in skimmed milk for 4 hrs at 30°C and cooled in the refrigerator at 4°C. Sterilized milk was finally inoculated with a mixed ratio of 50: 50 of the isolated lactic acid bacteria grown in skimmed
milk. The inoculated milk was incubated for 24 hrs at 30°C and cooled at 4°C before using as a starter culture.

**Physical analysis (rheological testing)**

**Viscosity (cP)**
The viscosity was determined using Brookfield DV-11+ Viscometer (model No. M/03-163-A0404 Middleborough, Massachusetts, U.S.A). Spindle no. 4 was used at speed of 10 rpm and the reading on the viscometer was taken after 1 minute for each sample (Moyane and Jideani, 2013).

**Syneresis**
The syneresis was determined using a Harmonic Series Centrifuge (model no. PLC-O12). Measured 40g of the sample was put in a centrifuge tube and set the centrifuge at 3500 rpm for 10 minutes and took the readings by decanting the supertant after centrifuging in a measuring cylinder and the percentage calculated.

**Physiochemical analysis**

**pH**
The pH of the samples was determined using pH meter, (EUTECH instruments pH 700, pH/mV/OC/OF meter, model no. ECPH 700425, Auckland, New Zealand). The pH and temperature probe were immersed in the sample. The instrument was calibrated using standard buffer solutions at pH 4 and 7 before testing the samples.

**Acidity (titration for lactic acid concentration)**
Acidity of the samples was determined by measuring 10mL of emasi sample in a beaker, adding 3-5 drops of phenolphthalein indicator into the sample and titrating using 0.1N of sodium hydroxide (NaOH) until the sample changed to a light pink colour. The volume of the NaOH used to titrate each sample was recorded. The percentage of lactic acid was calculated using the equation (NZIFST, 2010):

\[
\text{Acid} \%(%) = \frac{(\text{ml of NaOH used}) \times (\text{concNaOH})(0.096 \text{ milli equivalent weight of lactic acid})}{\text{Weight of sample}}
\]

**Sensory evaluation**
The sensory evaluation analysis was conducted using the acceptance test at UNESWA Luyengo campus. Assessors were randomly selected so as to take part in the study. Respondents were informed of the study before answering questions. To evaluate sensory parameters a structured questionnaire was used to capture information and people’s reaction towards the different products based on a 7-point hedonic scale. The attributes evaluated include taste, appearance, texture, aroma and overall acceptability. The rating scores used were as follows:

- Like very much=7
- Like moderately=6
- Slightly like=5
- Neither like nor dislike=4
- Slightly dislike=3
- Moderately dislike=2
- Very much dislike=1

**Statistical analysis**
The data collected from the study was subjected to three-way analysis of variance (ANOVA) using statistical software Statistix© (Statistix, 2006). Where means were significantly (P < 0.05) different, least significant difference (LSD) was used to separate means at 95% confidence level.

**RESULTS**

**Physiochemical properties of emasi**

**Effect of bacterial culture, fermentation time and inoculum size on physiochemical properties of emasi**

**Titratable acidity (%)**
The effect of commercial lactic culture, isolated *Leuconostoc* ssp. and *Lactococcus* ssp., mixed culture from traditional *emasi* and mixed culture from *emasi* sorghum meal on titratable acidity of *emasi* were significantly (P < 0.05) different. Mixed culture from *emasi* sorghum meal had the highest titratable acid content of 1.22% whilst the commercial lactic culture had the lowest content of 0.96% (Table 1). The effect of fermentation time at 12, 18 and 24 hours on titratable acid were all significantly different (P<0.05). At 24 hours the highest titratable acid percentage of 1.18% was recorded whilst at 18 hours it was 1.10%. The lowest titratable acid was recorded at 12 hours 1.01% (Table 1). The effect of inoculum size on titratable acidity was not significantly (P<0.05) different at 2.5% and 5% (Table 1). However, at 10% inoculum size the mean was significantly (P<0.05) different from the others.

**pH**
The effect of commercial lactic culture, isolated *Leuconostoc* ssp. and *Lactococcus* ssp., mixed culture from traditional *emasi* and mixed culture from *emasi* sorghum meal on pH of *emasi* were significantly (P<0.05) different among the treatments. The commercial lactic culture had the highest pH of 4.26 whilst the isolated *Leuconostoc* ssp. and *Lactococcus* ssp. culture (4.18) and mixed culture from traditional *emasi* (3.99) had lower readings (Table 1). The mixed culture from *emasi* sorghum meal recorded the lowest pH reading of 3.87. The effect of fermentation time on pH was not significantly (P>0.05) different at 18 hours (4.07) and 24 hours (3.99) (Table 1). However at 12 hours (4.15) there was a significant difference (P<0.05) with 24 hours (3.99). There was no significant (P>0.05) difference in the effect of inoculum size on pH of *emasi* (Table 1).

**Viscosity**
The effect of commercial lactic culture, isolated *Leuconostoc* ssp. and *Lactococcus* ssp., mixed culture from traditional *emasi* and mixed culture from *emasi* sorghum meal on viscosity of *emasi* were significantly (P<0.05) different. Mixed culture from *emasi* sorghum meal had the lowest viscosity of 2222 cP whilst the mixed culture from traditional *emasi* (2798 cP) and the isolated *Leuconostoc* ssp. and
**Effect of bacterial culture...emasi**

**Lactococcus ssp** culture (3144 cP) had higher readings (Table 1). The commercial lactic acid culture produced the highest viscosity reading of 3551 cP. The effect of fermentation time on viscosity of *emasi* was significantly (P<0.05) different at 12 hours (3199cP) and 24 hours (2681cP). There was no significant (P>0.05) difference at 24 hours and 18 hours (Table 1). There was no significant (P>0.05) difference in the effect of inoculum size on viscosity of *emasi* (Table 1).

**Syneresis**

The effect of commercial lactic culture, isolated *Leuconostoc* ssp. and *Lactococcus* ssp., mixed culture from traditional *emasi* and mixed culture from *emasi* sorghum meal on syneresis of *emasi* were significantly different (P<0.05). The mixed culture from *emasi* sorghum meal had the highest syneresis percentage of 56.5% whilst the mixed culture from traditional *emasi* (55.5%) and commercial lactic acid culture (50.7%) had lower percentages (Table 1). The isolated *Leuconostoc* ssp. and *Lactococcus* ssp had the lowest syneresis percentage of 48.6%.

**Table1: Effect of bacterial culture, fermentation time and inoculum size on physiochemical properties of *emasi***

<table>
<thead>
<tr>
<th>Physiochemical qualities</th>
<th>Bacterial Culture</th>
<th>Fermentation time (hrs)</th>
<th>Inoculum size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COMM</td>
<td>ILLC</td>
<td>MCTE</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>4.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.99&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>3551&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3144&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2978&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Syneresis (%)</td>
<td>50.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different letter in each constituent are significantly (P<0.05) different.

Key: COMM is commercial lactic acid culture, ILLC is isolated *Leuconostoc* ssp. and *Lactococcus* ssp. culture, MCTE is mixed culture from traditional *emasi* and MCES is mixed culture from *emasi* sorghum meal.

The effect of fermentation time on syneresis was not significantly (P>0.05) different at 18 hours (52.8%) and 24 hours (54.0%) (Table 1). However, at 12 hours (50.3%) there was a significant (P<0.05) difference from 18 and 24 hours as the lowest syneresis percentage was recorded of 50.3%.

There was no significant (P>0.05) difference in the effect of inoculum size on syneresis of *emasi* at 2.5% and 5% (Table 1). However, there was significant (P<0.05) difference at 10% with the highest syneresis of 53.9%.

**Sensory attributes of *emasi***

**Effect of culture type on sensory attributes of *emasi***

There was no significant (P>0.05) difference in the effect of bacterial culture on appearance of *emasi* (Figure 1). There was however a significant (P<0.05) difference in the effect of bacterial culture on aroma of *emasi*. The mixed culture from traditional *emasi* (MCTE) had the lowest degree of liking of 4.49 for aroma, whilst the isolated bacterial culture (ILLC) had the highest score (5.34) of them all.

There was a significant (P<0.05) difference in the effect of bacterial culture on taste of *emasi*. The isolated *Leuconostoc* ssp. and *Lactococcus* ssp. Culture (ILLC) scored the highest degree of liking (5.36) whilst the mixed culture from traditional *emasi* (MCTE) scored the lowest (4.38) score as the panellist “slightly disliked” the taste (Figure 1).

The effect of bacterial culture on texture of *emasi* had a significant (P<0.05) difference, higher scores were recorded for commercial lactic culture (COMM) (5.14) and isolated *Leuconostoc* ssp. and *Lactococcus* ssp. The panellists “liked” Culture (ILLC) (5.39) for texture as compared to mixed culture from traditional *emasi* (MCTE) (4.54) and sorghum meal (MCES) (4.60) which the panellists “neither liked or dislike”.

In terms of overall acceptability of *emasi*, there was a significant difference (P<0.05) with the commercial lactic culture (5.43) and isolated *Leuconostoc* ssp. and *Lactococcus* ssp. culture (5.58) scoring higher as the mixed culture from traditional *emasi* (4.90) and sorghum meal (5.02).
Effect of bacterial culture on sensory attributes of *emasi*

There were no significant (P>0.05) differences in the effect of fermentation time on appearance, aroma, taste, and overall acceptability of *emasi*. However, there was a significant (P<0.05) difference in the effect of fermentation time on texture of *emasi*. Higher degree of liking scores were recorded at 18 hours (5.05) and 24 hours (5.00) compared to 12 hours (4.17) with the lowest scores.

**DISCUSSION**

The effect of culture on sensory attributes and physiochemical properties of *emasi*.

The effect of culture on physiochemical properties of *emasi* such as titratable acidity, viscosity, syneresis and pH showed significant differences between the bacterial culture species. The commercial lactic culture had the lowest titratable acid of 0.96% and the highest pH reading of 4.26. The pH value of the commercial culture was within the range of 4.22 and 4.30 recorded by Moyane and Jideani (2013), but the titratable value was a bit higher compared to what they recorded. The organic acid (predominantly lactic acid) causes the pH of sour milk to drop (Gadaga *et al.*, 2004).

The lactic acid bacteria (LAB) that produce exopolysaccharides are often used to increase the viscosity of
stirred fermented milks, such as yoghurt and to decrease the susceptibility to syneresis (Dlamini et al., 2009). The commercial lactic culture had the highest viscosity reading of 3551 cP compared to other culture species and a low syneresis percentage of 50.7%. The value of viscosity of the culture is higher than that reported by Moyane and Jideani (2013), of 3330 to 1240 cP of commercial emasi products. Viscosity is influenced by the size of the particles and whey proteins from denaturation caused by pH (Masson et al., 2010). The syneresis of the sample was very high compared to that of 24.7% to 25% recorded by Nsibande and Dlamini (2000). Syneresis can be minimized by using stabilizers to the fermentation media or by increasing the total solids using milk powder (Schellhass and Moris, 1985). The sample was prepared without a stabilizer hence that is why the syneresis was high. Syneresis is also increased if the milk is not homogenised prior to fermentation (Schellhass and Moris, 1985). The sample was not homogenized hence the high syneresis percentage.

The isolated Leuconostoc mesenteroides ssp. mesenteroides/ dextranicum and Lactococcus lactis ssp. lactis had higher titratable acid of 1.04% (v/v) as compared to the commercial lactic culture and a lower pH reading of 4.18. The isolated Leuconostoc ssp. and Lactococcus ssp. culture had the lowest syneresis percentage compared to other cultures (commercial lactic culture, mixed culture from traditional emasi and emasi sorghum meal). This can be due to their ability to produce exopolysaccharides thus increasing viscosity and reducing syneresis (Dlamini et al., 2009). Syneresis or whey separation is not desirable in cultured dairy products because it indicates an inferior product (Nsibande and Dlamini, 2000). Isolated yoghurt starter can be used in yoghurt manufacturing in an industrial large scale (Bashiti, 2010). The isolated Leuconostoc ssp. and Lactococcus ssp. culture was as good as the commercial lactic culture. The use of pure cultures enables different acid flavours to be developed leading to a range of fermented (cultured) products (Kurmann et al., 1992).

The mixed culture produced emasi had a titratable acid of 1.17% and a very low pH value of 3.99 as compared to the emasi produced using the commercial culture and the isolated bacterial strains culture. The low pH in fermented milk offers a selective environment for yeast growth, but is unfavourable for some bacteria (Fleet, 1990) and spoilage may become evident when the yeast population reaches 10⁵ to 10⁶ cells/g (Fleet, 1990). Syneresis is high when acidity is high (Nsibande and Dlamini, 2000). A low viscosity reading of 2796 cP was recorded in this emasi and this though, was within the range of Moyane and Jideani (2013) but lower than that of the emasi from the isolated Leuconostoc ssp/ Lactococcus ssp. culture and commercial lactic culture. However, the syneresis was higher as compared to that of the commercial and isolated bacterial strains culture.

The mixed culture from emasi sorghum meal had the highest titratable acidity of 1.22% and also the lowest pH value of 3.87. According to Sanet-Bali et al. (2012), traditional fermented milks are considered safe because of the low pH and the production of antimicrobial substances by fermenting organisms. The syneresis percentage of the mixed culture from sorghum meal was the highest of them all which was 56.5% and the viscosity was also low at 2222cP. High syneresis results in low viscosity due to the high whey content of the product. This could be attributed to the fact that the culture is mixed and undefined.

Sensory evaluation

The sensory scores indicated that the consumers “liked” the appearance of all bacterial culture species presented as emasi. In terms of aroma the panellists neither liked nor disliked the mixed culture from traditional emasi and sorghum meal emasi, however the commercial lactic culture and the isolated bacterial strains were ranked higher with panellists “liking” the culture. The aroma, in general, of dairy products is complex due to the heterogeneous nature of milk (Moyane and Jideani, 2013).

In terms of taste of the culture, the isolated bacterial strains and commercial lactic culture species were both ranked higher showing that the consumers “liked” them whilst the two mixed culture from traditional emasi and sorghum meal where ranked lower as the panellists “slightly disliked” the culture species. The two mixed culture from traditional emasi and sorghum meal’s taste scores might have been influenced by the high acidic content of the samples.

In a study done by Moyane and Jideani, (2013), samples which had low viscosity also recorded lowest consumer acceptance on texture and smoothness sensory attributes. The texture of isolated Leuconostoc ssp. and Lactococcus ssp. culture had the highest scores where the panellists “liked” the product; this can be due to the high viscosity reading and very low syneresis percentage. The commercial lactic culture also had high sensory scores. The two mixed culture from traditional emasi and sorghum meal had low texture scores from the panellists, this can be due to the high syneresis percentages and low viscosity readings. The overall acceptability of both the commercial lactic culture and isolated bacterial strains had high scores as the panellists “liked” the culture species compared to the two mixed culture from traditional emasi and sorghum meal which had low score as the panellists “slightly disliked” them.

The effect of fermentation time on sensory attributes and physiochemical properties of emasi

The effect of fermentation time at 12hrs, 18hrs and 24hrs on titratable acidity were all significantly (P<0.05) different. At 12hrs the titratable acid percentage was low at 1.01% and gradually increasing at 18hrs and 24hrs being the highest at 1.18%. The pH values are also noticed to be decreasing as
the fermentation time increase. pH and titratable acid percentage influence syneresis and viscosity. As the pH decrease and T.A increase the syneresis increased but at 18hrs and 24hrs there was no significant (P<0.05) difference. The viscosity decreased due to the increase in syneresis from 12hrs, 18hrs and 24hrs with readings as follows: 3199 cP, 2805 cP and 2681 cP. Eissa et al. (2010) stated that prolonged metabolic activity of micro-flora in yoghurt causes changes in the micro-structure of the media and hence affecting viscosity.

Sensory evaluation
The sensory score indicated that fermentation time did not have an effect on the appearance, aroma, taste and overall acceptability of emasi. The panellists liked the emasi regardless of the fermentation time. However, fermentation time had an effect on the texture of the product. Low scores were given at 12 hrs with panellists slightly disliking it whilst at 18 hrs and 24 hrs the panellists liked the texture.

The effect of inoculum size on sensory attributes and physiochemical properties of emasi
The effect of inoculum size (2.5%, 5% and 10% v/v) on bacterial culture species had no significant difference on viscosity and pH. However there was a significant difference on T.A and syneresis of emasi. The titratable acid at 2.5% and 5% were not significantly (P<0.05) different but at 10% the percentage was high at 1.15%, this affected the syneresis also with 10% inoculum size having the highest syneresis percentage.

The inoculum size had no effect on appearance, taste, texture and overall acceptability as the panellists liked all the inoculum sizes. However, on aroma there was a significant difference with 10% recording low sensory scores as the panellists “slightly disliking”. According to Moyane and Jideani, (2013), aroma of dairy products is complex due to the heterogeneous nature of milk. The significant difference in aroma does not affect the overall acceptability of the product.

CONCLUSION
The outcome of the study showed that the type of culture used to ferment milk influenced consumer acceptance of emasi compared to inoculum size and fermentation time. The commercial lactic culture and isolated Leuconostoc ssp. and Lactococcus ssp culture had higher viscosity readings and low syneresis percentages whilst the two mixed culture species from traditional emasi and sorghum meal had low viscosity readings and high syneresis percentages. The sensory evaluation indicated that consumers prefer the commercial lactic culture and isolated Leuconostoc ssp. and Lactococcus ssp culture species as compared to the two mixed culture from traditional emasi and sorghum meal. The inoculum size and fermentation time did not have any significant effect on the physiochemical properties and consumer preference of emasi. The best culture species from the study were the commercial lactic culture and the isolated Leuconostoc ssp. and Lactococcus ssp culture. The inoculum size best for fermenting is the 2.5%, there was no significant difference between the levels inoculum size, I would advise the lowest size as it is economic when fermenting emasi at commercial level. 18-24 hours would be the best time for fermenting emasi as during that time the mesophilic bacteria fermenting the milk have coagulated the media to a desired texture and taste preferred by the consumers.

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