Comparative Study on the Use of Transglutaminase Enzyme in Making Labneh from Different Kinds of Milk

Reham k. Elmenawy1*, Y. I. Abd-Elkader1, M. M. Elmetwally1 and Ola M. A. K. Shalabi2

1Animal production research institute, Agricultural Research Center, Dokki, Egypt
2Dairy Department, Faculty of Agriculture, Mansoura University

ABSTRACT

Present research aims to make labneh from different kinds of milk (buffalo, cow and goat) to compare the effect of the addition of transglutaminase (TG) on the examined treatments. Results show that the addition of TG did not considerably affect the development of acidity during the fermentation process, while the increase of acidity in B and C was faster than in G. On the other hand, an increase in the yield of labneh made with added enzyme compared with samples without TG from was 43.16 to 50.55%, 38.78 to 48.16 % and 22.50 to 37.20%, in the same order. In addition, an improvement in the gel strength and decrease in the syneresis was observed. The chemical composition and sensory properties was determined during 21 days showed an increase in the total solid (TS), total protein (TP) and total volatile fatty acid (TVFA) in the samples treated with TG, while there was no noticeable effect on acidity and pH. The addition of TG in all of the examined samples led to improvement in the sensory value and access to creamy body, particularly in the labneh made from cow milk with added TG.

Keywords: Transglutaminase, Gel strength, Syneresis, Total volatile fatty acid.

INTRODUCTION

Labneh or concentrated yogurt is fermented milk in the Middle East having an acidic flavor and milky white color. Labneh usually characterized with softness, smoothness, and spreadability. It made by using strains of lactobacillus delbrueckii subsp bulgaricus and streptococcus thermophilus (Shamsia 2012).

Transglutaminases(EC2.3.2.123) (TG) are enzymes that stimulate forming an isopeptide bond between γ-carboxamide groups (\(-\text{C}=\text{O}\)NH2) of glutamine residue side chains and the ε-aminic groups (NH2) of lysine residue side chains with subsequent release of ammonia (NH3) naturally. Lysine and glutamine residues must be bound to a peptide or a protein to happen this cross-linking (between separate molecules) or intramolecular (within the same molecule) reaction. Bonds formed by transglutaminase show high resistance to proteolytic degradation (proteolysis). (Dejong and Koppelman 2002 and Griffin et al., Truong et al., 2004).

Recently, the interest in improving the protein properties of food products has received great attention. Transglutaminase is one of the most important methods to modify the properties of protein in food and is one of the enzymes that stimulates an acyl transfer reaction in the existence of Ca2+(Folk 1983). This reaction between ε-carboxaminic group of peptide - bound glutamine (acyl donors) and primary amino groups in variety of amino compounds (acyl acceptors) as lysine which results in curse (E-(Y-glutamyl)lysine (Aeschlimann and Paulsson 1994; Soawes et al., 2004; Truong et al., 2004; El nawawy et al., 2009). This denaturation why protein makes their amino acid available for TGM (Abou mahmound and Savellio1990; Traore and Meunier 1992).

Treatment of dairy products with microbial transglutaminases (MTG) led to improving the functional properties, flavour, viscosity, solubility, serum holding capacity, gel firmness and less allergen proteins (Ozren, 2006; Lee and Chin 2010; Fernando and Susan 2012).

The best result was obtained from addition of microbial transglutaminases (MTG) on yoghurt milk was 0.04% for 120 min setting at 40°C resulting in enhancing the functional properties of yogurt (Aproduetal., 2012).

MATERIALS AND METHODS

Fresh raw goat, cow, and buffalo milk was obtained from El-Serw Animal Production Research station, Animal Production Research Institute, Agriculture Research center, Egypt. Starter of commercial classic yogurt containing streptococcus thermophiles and lactobacillus delbrueckii subsp bulgaricus (1:1) was obtained from Chr. Hansen. lab A/S Copenhagen, Denmark. Transglutaminase (MTGase) Activa TG-I was bought from Ajinomoto (Incteane, NJ, U.S.A), the enzymatic product is consisted of 99% maltodextrin and MTGase with declared enzymatic activity of about 100 UE/g.

The chemical composition of differential used milk was presented in Table (1)

<table>
<thead>
<tr>
<th>Table 1. Chemical analyses of different milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of milk</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Goat</td>
</tr>
<tr>
<td>Cow</td>
</tr>
<tr>
<td>Buffalo</td>
</tr>
</tbody>
</table>

*Corresponding author.
E-mail address: gananabil2013@yahoo.com
DOI: 10.21608/jfds.2021.178951
For the production of labneh, milk samples were heated to 90 °C/10 min, followed by cooling to 40 °C. TG enzyme used in experiment was 0.04g MTGase/100g milk. Inoculation was carried out for 20 min, and the cross-linking reaction was stopped by thermally treating at 90°C for 2 min followed by cooling to 40°C. Inoculation with 2% yogurt starter was carried out until complete coagulation. Then it was put into cheese bags which were hung in the refrigerator room at 4°C overnight (12 h) to allow why drainage.

Total solids (TS %), titratable acidity (TA %) and fat contents of the different labneh samples were determined following the methods described in Ling (1963). Protein content was determined by the kjeldahl (AOAC 2000). Total volatile free fatty acids (TVFFA) was estimated as ml. 0.1N NaOH/10gm, and labneh samples was measured by using the method of Kosikowski (1982). The determination of pH in ten grams of the labnen water and was mixed to measure pH and was diluted with 70 ml distilled. The pH meter (Mettest, Toledo MP220, Switzerland) was calibrated with standard buffers at PH 4 and 7 (BDHL laboratory, England) prior to measuring the pH of the mixture.

The gel strength was measured at 4-6 °C by penetration measurements (Stevens-L.F.R.A Texture Analyssiser, CNS Farnell, Borehamwood, UK) the instrument was adjusted to the following conditions: cylindrical probe area 5.07 cm²; penetration speed 1.0 mm/s; penetration distance , 20 mm into surface . The determination of Gel strength was done in triplicate and was showed as N/cm² of probe area. Synersis was estimated by the centrifugation procedure. Approximately 20 g of yogurt was transmitted to a 50 mL glass tube and was centrifuged at 3500 rpm for 15 min at 20 °C. It was measured as the released percentage whey over the initial gel weight and as an average of three determinations:

\[ \text{Synersis} \% = \frac{\text{weight of supernatant}}{\text{weight of yogurt}} \times 100 \]

Yield was calculated as follows:

\[ \text{Yield} \% = \frac{\text{weight of labneh}}{\text{weight of milk used to make labneh}} \times 100 \]

Statistical analysis of the obtained data were carried out as mean ± standard deviation of three replicates. Except for the data of texture and sensory evaluation that were analyzed using one-way ANOVA and the other data were statistically analyzed by SPSS statistics 22.0 using two-way ANOVA to evaluate the significant differences between the means of samples and storage period. The means of results were compared by the Tukey test at a significance level of 5% (p < 0.05).

**RESULTS AND DISCUSSION**

Results indicated in Figure (1) show the effect addition of MTG on the coagulation time of labneh. It is clear that the use of milk with MTG resulted in reduction in the fermentation time in all samples, which came in agreement with Abdulquadret al. (2014). MTGase with different doses significantly increase yoghurt pH value, compared to the untreated yoghurt. The use of MTG accelerates the gel-forming product, especially in goat milk that has long fermentation with fragile gel, which agrees with Aprodual et al. (2012), while disagrees with the results in samples in the absence of the enzyme obtained by Lorenzen et al., 2002; and Neve et al., 2001.

Data illustrated in Figures (2 and 3) appear that yogurt before putting into cheese bag show that the use of TGM resulted in significant increase in gel strength, compared with yoghurt without enzyme were 56.16, 45.72 and 47.76 %, respectively in buffalo, cow and goat, respectively, and on the contrary a significant decrease in why synersis was observed 1014.89, 18.18 and 24.24%, respectively, which came in agreement with Lorenzen et al. (2002).
Total protein content in labneh and whey illustrated in Figure 4 show that the addition of the transglutaminase enzyme resulted in an increase in the concentration of protein in curd over the amount of protein in whey. Transglutaminase catalyses an acyl transfer reaction between γ-carboxamide groups of peptide-bound glutamine residues (acyl donor) and the primary amino groups in many amine compounds (acyl acceptor) that includes peptide-bound ε-amino groups of lysine residues. Because of cross-linking of peptide-bound glutamine and lysine residues ε-(γ-glutamyl), lysine iso-peptide bonds and high-molecular weight polymers are composed. The nonexistence of amine substrates, transglutaminase is able to catalyze the deamidation and amine incorporation of glutamine residues (Souweida et al., 2004).

Table 3. Chemical analyses of labneh during 21 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>zero</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>means</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.6±1.0</td>
<td>3.6±1.0</td>
<td>3.7±1.0</td>
</tr>
<tr>
<td>BM</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.5±1.0</td>
<td>3.5±1.0</td>
<td>3.6±1.0</td>
</tr>
<tr>
<td>C</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.8±1.0</td>
<td>3.7±1.0</td>
<td>3.6±1.0</td>
</tr>
<tr>
<td>CM</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.6±1.0</td>
<td>3.6±1.0</td>
<td>3.5±1.0</td>
</tr>
<tr>
<td>G</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.6±1.0</td>
<td>3.6±1.0</td>
<td>3.5±1.0</td>
</tr>
<tr>
<td>GM</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.6±1.0</td>
<td>3.6±1.0</td>
<td>3.5±1.0</td>
</tr>
<tr>
<td>Means</td>
<td>3.8±1.0</td>
<td>3.9±1.0</td>
<td>3.6±1.0</td>
<td>3.6±1.0</td>
<td>3.5±1.0</td>
</tr>
</tbody>
</table>

As with the organoleptic properties of the examined labneh, it is clear from the results in Table 4 that labneh from treatment milk with TGM gained high score of organoleptic properties which cross linking of milk protein by TGM improved the sensory properties as flavour, texture and color (El nawawy et al., 2009; Aprodu et al., 2012; Dinkci 2012). Especially texture of labneh from goat milk (GM). The goats gel is weaker than cow’s milk gel (Ardelean et al., 2013) but the use of TGM was improved of the total sensory properties.

Table 4. Effect of enzyme addition on the organoleptic properties on labneh from different milk.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Flavour(50)</th>
<th>Texture(35)</th>
<th>Color(15)</th>
<th>Total(100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>46±1.0</td>
<td>31±1.0</td>
<td>14±1.0</td>
<td>91±1.0</td>
</tr>
<tr>
<td>BM</td>
<td>48±1.0</td>
<td>35±1.0</td>
<td>14±1.0</td>
<td>95±1.0</td>
</tr>
<tr>
<td>C</td>
<td>42±1.0</td>
<td>29±1.0</td>
<td>12±1.0</td>
<td>83±1.0</td>
</tr>
<tr>
<td>CM</td>
<td>47±1.0</td>
<td>34±1.0</td>
<td>14±1.0</td>
<td>95±1.0</td>
</tr>
<tr>
<td>G</td>
<td>40±1.0</td>
<td>28±1.0</td>
<td>13±1.0</td>
<td>81±1.0</td>
</tr>
<tr>
<td>GM</td>
<td>43±1.0</td>
<td>32±1.0</td>
<td>14±1.0</td>
<td>89±1.0</td>
</tr>
</tbody>
</table>

Values are described in means ± Standard Deviation (SD) of three independent replicates. Means in the same columns with different superscripts are significantly different (P<0.05), labneh were B, C and G labneh from milk without enzyme. BM, CM and GM labneh from milk with enzyme.
CONCLUSION

Enzymatic treatment of milk with TGase accelerated the gelling product especially goats milk, and led to significant higher in yield of labneh inaddition of increased gel strength and less syneresis. The enzymatic cross-linking reaction led to improve the rheological properties.

REFERENCES


