Influence of Addition of Egyptian Grape Seed Extract on the Antioxidant, Antibacterial Activities and Shelf Life of Traditional Labneh

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ABSTRACT

This study aimed to evaluate the impact of adding Egyptian grape seed (Red Romi) extract (GSE) to traditional Labneh in order to enhance its shelf life and functionality availability. Labneh was divided into four treatments; the first one served as control (with no GSE), the second, third and fourth treatments contained 0.05, 0.1 and 0.2% of GSE, respectively. During cold storage period, levels of titratable acidity increased, while levels of moisture decreased in all treatments. Addition of GSE resulted in an increase of the antimicrobial and antioxidant activities of crude extract of Labneh. Also, Labneh samples fortified with GSE had more shelf life than control. GSE did not negatively effect on the viability of starter culture. Labneh fortified with 0.2% GSE showed the lowest score for its appearance.

Key words: Antimicrobial, Antioxidant, Grape seed extract, Labneh, Shelf life, Sensory properties.

INTRODUCTION

Labneh (concentrated yoghurt) is the most popular fermented milk in Middle East and it is traditionally manufactured from yoghurt after removal of part of its whey (Tamime and Robinson, 1999). Due to poor sanitation conditions of Labneh that usually associated with cloth bags used in its manufacture, the shelf life of Labneh is short (El-Samragy, 1997) and Off-flavors with undesirable physicochemical changes occurred (Muir and Banks, 2000). Therefore, addition of preservatives or antimicrobial agents were necessary in order to increase the shelf life of Labneh (Draugbon, 2004). Natamycin (Tawalbeh et al., 2014), different essential oils e.g. thyme, marjoram and sage were used as preservatives (Al Otabi and El Demerdash, 2008). There is an increasing interest in utilization of fruit processing wastes like Grape seed extract (GSE) as ingredients for preparing functional food. GSE as a functional ingredient contained several flavonoids with a phenolic nature such as flavanols (catechin and epicatechin), procyanidins and phenolic acids (Weseler and Bast, 2017). It has many beneficial effects including antioxidant (El-Beshbishy et al., 2009; Krithika et al., 2005), anti-inflammatory activities (Cádiz-Gurrea et al., 2017), hypcholesterimic (Cetin et al., 2008) and anti diabetic effects (Montagut et al., 2010) and hepatoprotective agent (Wang et al., 2015). Furthermore, it is used as food additives and as antimicrobial agent in Japan (Yamakoshi et al., 2002). Also, fortification of yoghurt with GSE resulted in an increase in its phenolic content and antioxidant capacity (Karaaslan et al., 2011). Therefore, this study aimed to evaluate the impact of utilization of Egyptian type of GSE on some properties and shelf life of Labneh during the period of cold storage.

MATERIALS AND METHODS

Chemicals and reagents

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical and ethanol were purchased from Merck, Darmstadt, Germany. Cow milk was purchased from Dairy Unit, Faculty of Agriculture, Cairo University. Freeze dried yoghurt culture YC-Flex 1 was purchased from CHR-Hansen, Copenhagen, Denmark. Bacillus (B.) subtilis NRRL-B-356, Escherichia (E.) coli NRRL-B-409 and Staphylococcus (Staph.) aureus NRRL-B-120 as indicator bacterial strains were obtained from Northern Regional Research Laboratory (NRRL), Peoria, Illinois, USA.

Preparation of GSE

Local type of Egyptian GSE (Red Romi) were dried in drying oven at 50°C for 3 days. GS ground to fine powder by grinder (Moulinex, model 721, Paris, France). GSE was prepared according Hansen (1995).

Propagation of indicator bacterial strains

The indicator bacteria strains B. subtilis NRRL-B-356; E. coli NRRL-B-409 and Staph. aureus NRRL-B-120 were propagated in Nutrient broth medium (Oxoid, Hampshire, UK) and incubated at 32°C for 24 h.
Manufacture of Labneh

Fresh cow’s milk (Fat 3.2%, total solids 11.70 %) was heated at 90°C for 20 min, cooled to 45°C. The homogenized milk was divided to four treatments which were mixed individually with the GSE at 0, 0.05, 0.1 and 0.2 %, respectively from control, T1, T2 and T3 followed by inoculation with 0.02% of freeze Dried yoghurt culture. The milk was incubated at 40°C for 3 h until complete coagulation. The resultant coagulant was mixed thoroughly with 0.5% NaCl. Samples were taken for analysis either fresh (day 0) or during the thirty days of cold storage (Robinson and Tamime, 1994).

Identification of phenolic compounds in grape seed

Profiles of Phenolic Compounds in grape seed extract were identified in Grape seed extract by HPLC as described by Corrales et al. (2009).

Changes in titratable acidity and moisture content during cold storage

Titratable acidity and moisture of Labneh samples was measured according to (A.O.A.C, 2007).

Viability of starter culture during cold storage

Viable count of starter culture at the end of fermentation and during 30 days of cold storage (5±1°C) was enumerated using MRS (Merck, Darmstadt, Germany) for total Lactobacillus delbrueckii subsp bulgaricus and M17 (Oxoid, Hamshire, UK) for Streptococcus thermophilus.

Antioxidant activity

The antioxidant activity of crude extract of Labneh was measured as a scavenging activity of the free radical DPPH as previously described by Lee et al. (2004).

Antibacterial activity

The antibacterial activity of crude extract of Labneh was carried out using well diffusion method against B.subtilis NRRL-B-365, E. coli/NRRL-B-409 and Staph. aureus NRRL-B-120 (Varadaraj et al., 1993).

Microbiological quality of Labneh during cold storage

Labneh samples were microbiologically analysed using pour plate technique for enumerating total aerobic mesophilic and psychrotrophic bacteria and molds and yeasts according Al Otaibi et al. (2008).

Organoleptic properties

Samples of Labneh were organoleptically scored for flavor (50 points), body and texture (40 points) and appearance (10 points) according to score card suggested by Keating and Randwhite (1990).

Statistical analysis

Results were presented as mean values and standard deviation.

Table 1: Profiles of Phenolic Compounds in Grape seed extract.

| Compound            | Conc. mg/kg |
|---------------------|-------------|
| Quinol              | 1.10        |
| Gallic              | 1.11        |
| Catechol            | 5.3         |
| p-Hydroxy benzoic   | 18.45       |
| Caffeine            | 8.65        |
| Chlorogenic         | 11.03       |
| Vanillic acid       | 9.15        |
| Caffeic acid        | ND          |
| Syringic acid       | 5.88        |
| Vanillin            | 0.48        |
| p-Coumaric acid     | 0.33        |
| Rutin               | 11.16       |
| Ellagic acid        | 48.84       |
| o-Coumaric acid     | 2.15        |
| Salicylic acid      | 4.21        |
| Myricetin           | 19.91       |
| Quercitin           | 15.95       |
| Rosemarinic         | 15.08       |
| Neringein           | 25.91       |
| Kamphorol           | 6.83        |

Table 2: Mean values of titratable acidity and moisture content in Labneh during cold storage (5±1°C).

| Treatments*          | Storage period, days | Titratable acidity, % lactic acid | Moisture% |
|----------------------|----------------------|-----------------------------------|-----------|
| Control              | 0                    | 0.85±0.12c                        | 73.05±0.25a|
|                      | 7                    | 0.90±0.10bc                       | 72.90±0.31a|
|                      | 15                   | 1.00±0.15b                        | 72.40±0.15b|
|                      | 30                   | 1.35±0.12a                        | 71.80±0.26c|
| Treatment 1          | 0                    | 0.87±0.17c                        | 72.90±0.25a|
|                      | 7                    | 0.93±0.20bc                       | 72.80±0.22a|
|                      | 15                   | 0.95±0.16bc                       | 72.30±0.20b|
|                      | 30                   | 1.40±0.14a                        | 71.70±0.25c|
| Treatment 2          | 0                    | 0.86±0.12c                        | 73.01±0.23a|
|                      | 7                    | 0.97±0.15bc                       | 72.81±0.31a|
|                      | 15                   | 1.10±0.13b                        | 72.05±0.40b|
|                      | 30                   | 1.38±0.11a                        | 70.25±0.35c|
| Treatment 3          | 0                    | 0.83±0.12c                        | 72.91±0.23a|
|                      | 7                    | 0.92±0.15bc                       | 72.60±0.28a|
|                      | 15                   | 1.15±0.14b                        | 71.65±0.30c|
|                      | 30                   | 1.42±0.15a                        | 70.95±0.32c|

*: Control: Labneh was manufactured without addition of grape seed extract, Treatment 1: Labneh was manufactured with addition of 0.05% grape seed extract to the milk, Treatment 2: Labneh was manufactured with addition of 0.1% grape seed extract to the milk, Treatment 3: Labneh was manufactured with addition of 0.2% grape seed extract to the milk. Statistic means for letters, significant.
deviation (SD) of the three replicates which were calculated by using Excel Microsoft Office Ver.2010. Tukey test was applied for evaluating the significant differences between different parameters SAS Ver. 4.02 (2000).

RESULTS AND DISCUSSION

Results in Table 1 shows the profile of different phenolic compounds in GSE using HPLC. It can be noticed that the Ellagic acid and Neringein concentrations were the highest. The present results were not in line with Chouchouli et al., (2013) whereas Gallic acid, Epicatechin and Catechin were the most predominant polyphenols in GSE. The differences in profile of phenolic compounds in GSE were attributed to the difference in the type of GSE, conditions of storage and methods of extraction and freeze drying.

Results in Table 2 revealed that moisture contents (%) in different Labneh samples decreased during thirty days of cold storage period. The decrease was higher in Labneh fortified with 0.1(T2) and 0.2% (T3) than control (C) and Labneh samples fortified with 0.05%GSE at the end of

### Table 3: Mean value of counts (Log CFU/g) of starter culture in Labneh during cold storage (5±1°C).

| Treatments* | Storage period, days | Lb. delbrueckii subsp bulgaricus | S. thermophiles |
|-------------|----------------------|---------------------------------|-----------------|
| Control     | 0                    | 8.30±0.30a                      | 8.10±0.40a      |
|             | 7                    | 8.20±0.20a                      | 8.20±0.30a      |
|             | 15                   | 8.20±0.60a                      | 8.10±0.30a      |
|             | 30                   | 7.50±0.50b                      | 7.20±0.40b      |
| Treatment 1 | 0                    | 8.10±0.60a                      | 8.20±0.70a      |
|             | 7                    | 8.20±0.80a                      | 8.30±0.52a      |
|             | 15                   | 8.25±0.65a                      | 8.25±0.71a      |
|             | 30                   | 7.65±0.42b                      | 7.25±0.35b      |
| Treatment 2 | 0                    | 8.20±0.62                       | 8.30±0.73a      |
|             | 7                    | 8.15±0.71a                      | 8.25±0.68a      |
|             | 15                   | 8.30±0.50a                      | 8.10±0.40a      |
|             | 30                   | 7.40±0.40b                      | 7.10±0.32b      |
| Treatment 3 | 0                    | 8.30±0.68a                      | 8.12±0.62a      |
|             | 7                    | 8.20±0.78a                      | 8.25±0.71a      |
|             | 15                   | 8.35±0.77a                      | 8.10±0.62a      |
|             | 30                   | 7.30±0.65b                      | 7.10±0.52b      |

*: Control: Labneh was manufactured without addition of grape seed extract, Treatment 1: Labneh was manufactured with addition of 0.05% grape seed extract to the milk, Treatment 2: Labneh was manufactured with addition of 0.1% grape seed extract to the milk, Treatment 3: Labneh was manufactured with addition of 0.2% grape seed extract to the milk. Statistic means for letters, significant. ±: Standard deviation.

### Table 4: Antioxidant Activity of crude extract of Labneh during cold storage (5±1°C).

| Treatments* | Storage period, days | Scavenging activity % |
|-------------|----------------------|-----------------------|
| Control     | 0                    | 38.05±1.01g           |
|             | 7                    | 39.05±1.87g           |
|             | 15                   | 39.65±1.68g           |
|             | 30                   | 40.15±1.30g           |
| Treatment 1 | 0                    | 55.70±1.04e           |
|             | 7                    | 53.15±1.82e           |
|             | 15                   | 46.56±1.25g           |
|             | 30                   | 43.65±0.85g           |
| Treatment 2 | 0                    | 69.35±1.62c           |
|             | 7                    | 63.85±1.03d           |
|             | 15                   | 58.62±0.72d           |
|             | 30                   | 52.51±0.60f           |
| Treatment 3 | 0                    | 76.65±0.75b           |
|             | 7                    | 70.89±0.92c           |
|             | 15                   | 65.51±0.82c           |
|             | 30                   | 59.76±0.73d           |
| Unfermented milk | 0            | 32.51±0.85h           |
| Ascorbic acid | 91.56±1.10a         |

*: Control: Labneh was manufactured without addition of grape seed extract, Treatment 1: Labneh was manufactured with addition of 0.05% grape seed extract to the milk, Treatment 2: Labneh was manufactured with addition of 0.1% grape seed extract to the milk, Treatment 3: Labneh was manufactured with addition of 0.2% grape seed extract to the milk. Statistic means for letters, significant. ±: Standard deviation.
storage period because of increase of acidity as well as syneresis effect during cold storage. The current results were in accordance with Al Otaibi and El Demerdash (2008). Results for titratable acidity of Labneh were in line with Al Otaibi and El Demerdash (2008), Mohamed et al. (2016) and El-Shafei et al. (2018), as they showed gradual increase along the storage period and this effect could be attributed to the activity of starter culture.

Data in Table 3 showed the changes in viable count (Log CFU/g) of starter culture (L. delbrueckii subsp bulgaricus and S. thermophilus) in Labneh samples during cold storage. During fifteen days of cold storage, the viable

**Table 5**: Antibacterial activity* of crude extract of Labneh against *Staph. aureus, B. subtilis and E. coli* during cold storage (5±1°C).

| Treatments** | Storage period, days | *Staph. aureus* | *B. subtilis* | *E. coli* |
|--------------|----------------------|----------------|--------------|-----------|
| Control      | 0                    | 3.60±0.30e     | 2.80±0.40gh  | 1.50±0.30fg |
|              | 7                    | 4.30±0.20d     | 3.10±0.20g   | 1.80±0.40f  |
|              | 15                   | 4.70±0.30d     | 3.50±0.30g   | 2.30±0.40de |
|              | 30                   | 5.40±0.30c     | 4.20±0.40f   | 2.40±0.20de |
| Treatment 1  | 0                    | 3.80±0.34e     | 3.10±0.31g   | 2.50±0.25d  |
|              | 7                    | 4.50±0.30d     | 3.50±0.25g   | 2.80±0.31d  |
|              | 15                   | 5.25±0.30c     | 4.20±0.41f   | 3.20±0.50bc |
|              | 30                   | 6.01±0.40b     | 4.85±0.52e   | 4.00±0.30a  |
| Treatment 2  | 0                    | 4.70±0.61e     | 4.80±0.35e   | 2.90±0.41d  |
|              | 7                    | 5.10±0.27d     | 5.10±0.18de  | 3.50±0.55bc |
|              | 15                   | 5.80±0.62c     | 5.70±0.35c   | 3.70±0.65b  |
|              | 30                   | 6.50±0.54b     | 6.40±0.15ab  | 4.55±0.45a  |
| Treatment 3  | 0                    | 5.60±0.32c     | 5.30±0.35c   | 3.22±0.20bc |
|              | 7                    | 6.20±0.15b     | 5.60±0.28c   | 3.40±0.50b  |
|              | 15                   | 7.10±0.45a     | 6.00±0.20b   | 4.00±0.35a  |
|              | 30                   | 7.55±0.20a     | 6.80±0.20a   | 4.30±0.15a  |

* Mean values of inhibition zone (mm).

**Control: Labneh was manufactured without addition of grape seed extract, Treatment 1: Labneh was manufactured with addition of 0.05% grape seed extract to the milk, Treatment 2: Labneh was manufactured with addition of 0.1% grape seed extract to the milk, Treatment 3: Labneh was manufactured with addition of 0.2% grape seed extract to the milk. Statistic means for letters, significant. ±: Standard deviation.

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count of starter culture was relative stable. On the other hand, at the end of storage period, the viability of yoghurt culture decreased in all treatments. These results indicated that GSE did not show any inhibitory effect on the growth of yoghurt culture as previously reported by Ersöz et al., (2011) in strained yoghurt and Karaaslan et al., (2011) in yoghurt and the decrease in viability of starter culture was due to cold storage and acidity in line with Mohamed et al., (2016).

The antioxidant activity, as DPPH free radical scavenging activity, of Labneh fortified with GSE was decreased during cold storage (Table 4) whereas ascorbic acid was used as positive control. Also, addition of different concentrations of GSE resulted in increased this activity in comparison with control and unfermented milk. The current results were in accordance with Yadav et al. (2018) who reported a decreased in DPPH scavenging activity in yoghurt sample fortified with GSE during fifteen days of cold storage period.

Antibacterial activities of crude extract of Labneh (with GSE), as shown in Table 5, was carried out against B. subtilis, E. coli and Staph. aureus which increased during the period of cold storage. Staph. aureus showed more sensitivity than B. subtilis and E. coli and control (without GSE). The increase of antibacterial activity of crude extract of Labenah might be attributed to increase of accumulation of metabolites produced by yoghurt culture that was in line with Hassan and Elshaghabee (2016). Moreover, Ersöz et al. (2011) noticed the antibacterial activity of GSE against E. coli ATCC 25922, Staphy. aureus ATCC 6538 and S. typhimurium.

Psychrotrophic bacteria cause sour and bitter taste in Labneh and the acidity and water activity of Labneh are favorable for growing molds and yeasts (Sahan et al., 2004). In control samples, psychrotrophic bacteria, molds and yeasts were detected at the end of cold storage. The range of log viable counts of psychrotrophic bacteria was 1.50-2.60 Log CFU/g and it was 1.20-2.50 Log CFU/g for molds and yeasts. On the other hand, fortification of Labneh with different concentrations of GSE resulted in inhibition growth of psychrotrophic bacteria and molds and yeast a long of storage period. These results may attributed to the antibacterial activity of used GSE as previously presented in Table 5. Results obtained by Mohamed et al. (2016) and Al Otabi et al. (2008) showed that fortification of Labneh with Papaya seed extract or thyme, marjoram and sage essential oil resulted in inhibition the growth of molds and yeast during cold storage of Labneh and enhanced the shelf life of Labneh.

Results of sensory evaluation for Labneh manufactured with 0.2 % GSE showed the lowest appearance score during cold storage period (Fig 1). Results obtained by Chouchouli et al. (2013), were close to the current results, as revealed that high level of supplementation of yoghurt with polyphenols extracted from GSE had negative effect on sensory evaluation of yoghurt.

**CONCLUSION**

In this research, Egyptian type (Red Romi) GSE as a source of polyphenols with antibacterial properties was used for enhancing some characteristics of traditional Labneh. Fortification of Labneh with 0.05 and 0.1% resulted in enhancing its shelf life as well as its antioxidant capacity without any antagonistic effect against the starter culture. On the other hand, fortification of Labneh with 0.2 % of GSE had negative effect on the appearance of Labneh. Therefore, our investigation recommend to use GSE with concentration ranged 0.5-0.1%.

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