ANTIOXIADANT ACTIVITY OF YOGHURT SUPPLEMENTED WITH NATURAL ADDITIVES

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Abstract: The aims of this study were the estimation of difference in physiochemical properties and antioxidant activity of yoghurts supplemented by different natural additives. In addition, the changes in physiochemical properties and antioxidant activity during five weeks storage were performed. The change in antioxidant activities was evaluated using the ABTS radical cations decolorizing assay. The statistical analysis showed that the yoghurt samples with the natural supplement have higher antioxidant activity than the plain yoghurt. In addition, the storage period affect the yoghurt properties by increasing its acidity and antioxidant activities by increasing the storage time. Furthermore, the plain and supplemented yoghurts are still consumable until the fifth week of storage but the favourable time to consume it with its higher antioxidant capacity is the 3rd week of storage.

Key Words: yoghurt, natural additives, antioxidant activity, Trolox equivalent

INTRODUCTION

Various reactive oxygen species such as hydrogen peroxide, superoxide and hydroxyl radical are known to cause oxidative damage not only to the food systems but also to living systems (Liu, Chen et al. 2005). The free radicals are the causative agent of many serious diseases in human such as cancer (Kinnula and Crapo 2004), cardiovascular diseases (Singh and Jialal 2006), neural disorder (Sas et al. 2007), Alzheimer's disease (Smith et al. 2000), Parkinson's disease (Bolton et al. 2000), aging (Hyun et al. 2006), and atherosclerosis (Upston et al. 2003). The natural antioxidants from food can protect the human body from the harmful effects of the free radicals, and they able to delay the progress of many chronic diseases (Liu et al. 2005). The natural antioxidants are preferable than the synthetic ones because some synthetic antioxidants have been reported to be carcinogenic (Liu et al. 2005).

Milk proteins considered the main source of the bioactive peptides that played a vital role in maintenance of antioxidant defence system (Gupta et al. 2009), the highest number of these peptides have been in the hydrolysates milk protein and fermented dairy products (Korhonen and Pihlanto 2006, Nagpal et al. 2011). Yoghurts are one of the most common fermented milk products and it is a worldwide food. It is traditionally consumed as a healthy food, because of their high nutritional values, health benefits, and its sensory properties (Gilliland 1989). Yoghurt is a coagulated milk produced by fermentation using the lactic acid bacteria, *Streptococcus thermophilus, and Lactobacillus Bulgaricus* (Gahruieet al. 2015). The fermentation process evolves the yoghurt antioxidant activity by releasing various bioactive peptides and free amino acids during lactic acid fermentation (Kudoh et al. 2001, Korhonen 2009). Yoghurts supplemented by different fruits,

vegetables, and other natural products to increase their antioxidant activity by providing more bioactive peptides and amino acids that act as a scavenger for the free radicals.

The aims of this study were to estimate the antioxidant activity and the pH of yoghurt supplemented with a different weight percentage (1, 3, and 5 wt%) of different natural additives that already known with their high antioxidant activity. Another aim of this study was to estimate the effect of storage time on the antioxidant activity and pH of the different yoghurt samples for five weeks storage in the fridge at 4 °C.

MATERIALS AND METHODS

pH measurement

The pH of the different yoghurt samples was measured according to *Zainoldin and Baba 2009* (Zainoldin and Baba 2009) using the pH meter (WTW 720 pH meter, Germany), by mixing 1 ml of yoghurt and 3 ml of distilled water . The measurements were performed weekly in triplicate during five weeks storage period to estimate the change in pH during the storage time.

Yoghurt samples preparation

The yoghurt samples were prepared at the Department of Food Technology laboratories at Mendel University, different types of natural additives with different weight percentage (1, 3, and 5 wt%) were added to yoghurt after fermentation process completion. The natural additives that used includes the Quinoa (Qu), Nopal (No), Apple fibres (Af), and Bamboo fibres (Bf), in addition to the plain yoghurt sample without any additives. All samples were stored at 4 °C and the pH and antioxidant activity were measured weekly to detect the changes.

Preparation of water-soluble extracts of the yoghurt samples (WSE)

The water soluble extract of the yoghurt samples was prepared according to *Perna et al. 2014* (Perna et al. 2014) with some modifications. Briefly, 50 ml of yoghurt samples were centrifuged at 9000 rpm (Hettich Universal 32 R centrifuge, Germany) at 4 °C for 15 min. The supernatant was filtrated separately through 0.45 µm membrane filter and the filtrates were stored at 4 °C for the analysis.

Antioxidant activity of ABTS radical scavenging system assay

The antioxidant activity of the different yoghurt samples was assayed according the method described by *Re et al. 1999* (Re et al. 1999). The ABTS (2, 2 – Azino- bis (3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical solution was created by oxidation of 7 mM ABTS solution with 140 mM potassium persulfate (K_2SO_4). The stock solutions of both reactants were prepared in ACS water and the ABTS radical solution was prepared by mixing 10 ml of 7 mM ABTS stock with 175 µl of the K_2SO_4 solution, and the mixture kept in the dark at room temperature for 12–16 hours before use. For estimation of the antioxidant activity, the ABTS radical solution was diluted with (96%) ethanol to get the absorbance of 0.700 ± 0.020 at 734 nm. Two millilitres of the diluted ABTS radical solution were mix with 100 µl of the yoghurt WSE and incubated at room temperature and the absorbance was measure after 30 min at 734 nm. The Trolox standard curve (0–2 mM) was created to calculate the Trolox equivalent antioxidant capacity (TEAC) for each sample and the results were expressed as µg TEAC/ml of yoghurt extract. The 96% ethanol was used as a blank in each assay, all the measurement was carried in triplicate and the percent of inhibition (%) was calculated using the following equation: Inhibition (%) = $[(A_{control} — A_{sample})/ (A_{control})] \times 100$

Statistical analysis

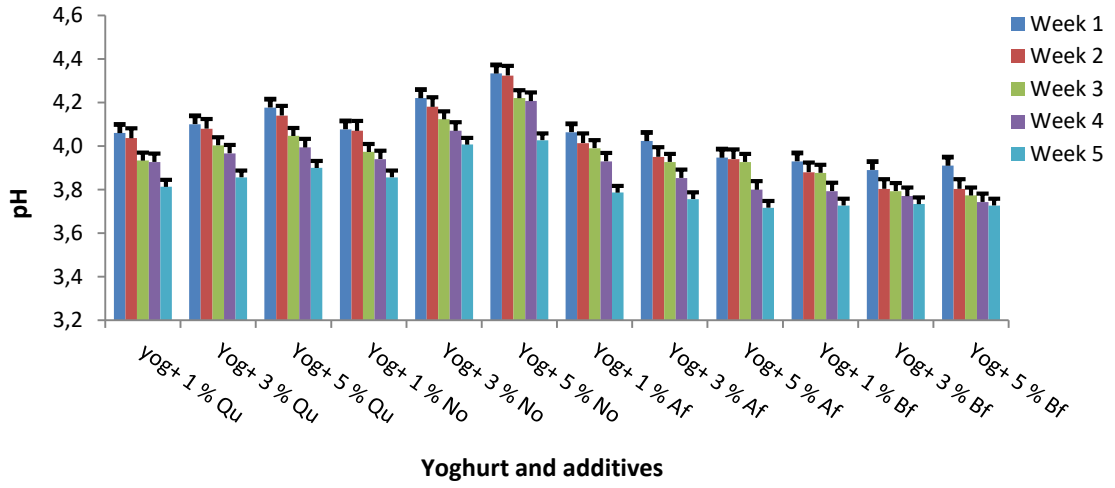
The result analysis performed by IBM SPSS Statistics 21 software. The one-way ANOVA was performed to detect the significance variations in pH, antioxidant activities, and TEAC between and within the yoghurt samples during the storage period at the significant level ($P < 0.05$). The correlation between the antioxidant activities and the additives percentage was estimated by Pearson correlation at the significant level ($P < 0.01$).

RESULTS AND DISCUSSION

Changes in pH during storage periods

The pH measurement considered a very sensitive tool to detect the acidity change of yoghurt during a refrigerated storage (Salij and Ismail 1983). In our study, the initial pH of all yoghurt samples during the first week was acidic and then its start to decline and be more acidic by increasing the storage time. The changes in pH values for the different yoghurt samples during the storage periods are shown in figure.1. The pH values were significantly ($P < 0.05$) decrease during the different storage periods in all yoghurt samples including the control. In addition, there was a significant difference ($P < 0.05$) in pH values between the different samples and within the same samples. The change in pH values for the yoghurt samples with the additives compared to the control related to the percentage and additives type, which affect the yoghurt taste and pH (Laye et al. 1993, Tarakçi and Kucukoner 2003). The continuous decline in pH of the yoghurt samples by increasing the storage time is related to the amount of lactic acid production because of acidification process, which is the main process during yoghurt fermentation (Aloğlu and Öner 2011, Nguyen and Hwang 2016). Furthermore, the continuous decrease in pH is considered an indicator for the high metabolic activities of the lactic acid bacteria which is the starter culture in almost all yoghurt types (Zainoldin and Baba 2009).

Figure 1 Change of pH of the yoghurts during different storage periods



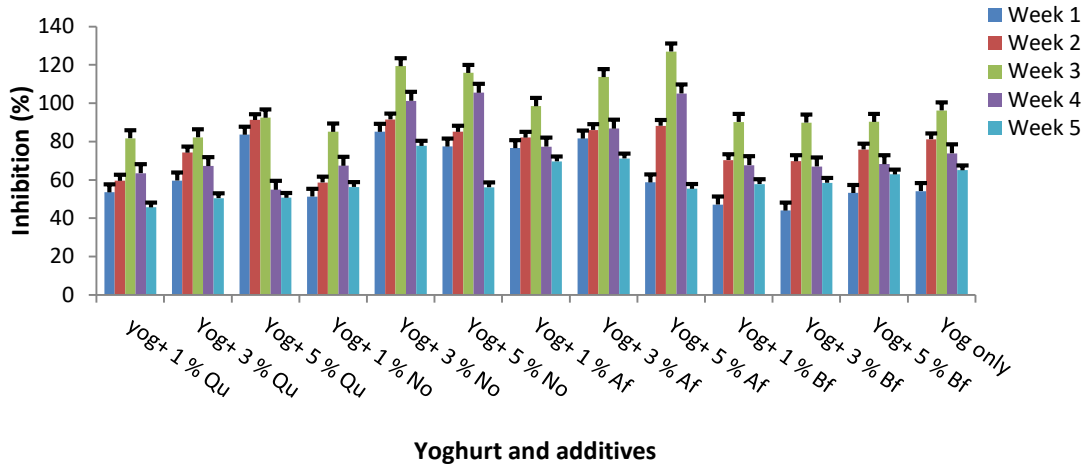
Legend: Yog– yoghurt, Qu – Quinoa, No – Nopal, Af – Apple fibre, Bf – Bamboo fibres

Change in Antioxidant activity of ABTS radical scavenger assay and Trolox equivalent antioxidant capacity (TEAC)

The most important feature of the antioxidants is their capability to trap the free radicals which are exist in the biological system from different sources (Aloğlu and Öner 2011). The free radicals may oxidize the macromolecules (nucleic acids, proteins and lipids) and can initiate a serious diseases (Zainoldin and Baba 2009, Srivastava et al. 2016). In our experiment, the yoghurt samples showed a significant variation ($P < 0.05$) in their antioxidant activities and the TEAC (Figure 2 and Figure 3) respectively. These variations between the different samples and within the sample with different percentage of the supplement are strongly related to the percentage and additives types especially there are many studies reported the antioxidant activities of our additives like Alvarez-Jubete et al. 2010, Wu et al. 2012, El-Mostafa et al. 2014. Generally, it has been reported that the natural additives enhance the antioxidant activity of the yoghurt and other foodstuff to evolve the consumer's health against the pathological process that are related to the free radicals (Pereira et al. 2013). Furthermore, the results showed that the antioxidant activity significantly ($P < 0.05$) increase with the storage time, it has reached the maximum value in the third week of storage and then start to decrease gradually for all samples including the control. The increase in antioxidant activity and TEAC equivalent are strongly related to the fermentation and post acidification during storage that determine the production of organic acids, and the decline in the antioxidant activity after the third week is related

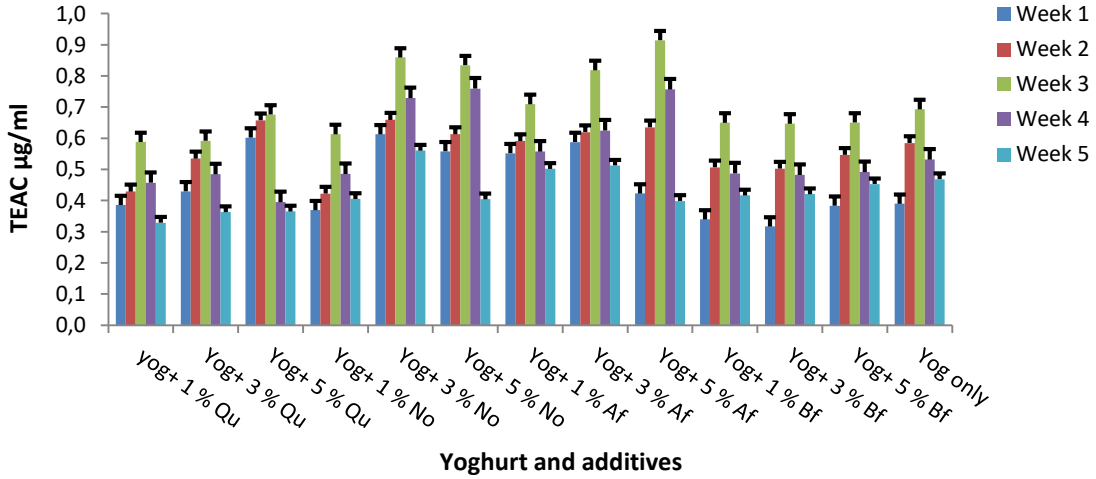
to the extensive proteolysis that produce smaller and less bioactive peptides (Correiaet al. 2005, Perna et al. 2014). Our results are in agreement with (Shori and Baba 2013, Perna et al. 2014, Shori and Baba 2014) who reported the increase in antioxidant activity of the yoghurt during the storage period and then start to decrease after the 21 days (3 weeks). Moreover, the results showed that the plain yoghurt without additives have also an antioxidant activity and its activity and the amount of TEAC improved by the additives. Our results are in agreement with different studies that reported the antioxidant activity of plain yoghurt like (Aloğlu and Öner 2011, Gjorgievskiet al. 2014, Yilmaz-Ersan et al. 2016). Furthermore, there was no significant ($P > 0.01$) correlation between the antioxidant activity and the additives amount in spite of different studies like (Prior et al. 1998, El Samh et al. 2013) reported the increase of antioxidant activity of yoghurts or foods by increasing the amount of additives.

Figure 2 Percentage change of antioxidant activity of yoghurts during storage periods



Legend: Yog– yoghurt, Qu – Quinoa, No – Nopal, Af – Apple fibre, Bf – Bamboo fibres

Figure 3 Change in Trolox equivalent capacity (TEAC) of yoghurts during storage periods



Legend: Yog– yoghurt, Qu – Quinoa, No – Nopal, Af – Apple fibre, Bf – Bamboo fibres

CONCLUSION

This study shows the effect of storage on the physiochemical properties and antioxidant activity of plain yoghurt supplemented with different natural additives. The results showed that the plain yoghurt has antioxidant activity but this activity can be improved by these supplements which are known for their antioxidant activities. Furthermore, the study showed that the storage conditions have an effect on the physiochemical properties of yoghurt like increasing the acidity, probably due to the increase of lactic acid production. Moreover, increasing the storage period of yoghurts improve

their antioxidant activity regardless if they have additives or not, the progress fermentation during the storage results in production of peptides and free amino acids that enhance the yoghurt antioxidant properties. In addition, based on our results we can say that the proper storage time of the yoghurt is 3 weeks (21 days), after this period the yoghurt is still acceptable to use by the consumers but it will be less quality considering our results that related to pH and antioxidant activity changes during the storage period.

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