EFFECT OF ANTIOXIDANTS ADDITION IN COMBINATION WITH MILK RE PASTEURIZATION ON THE PHYSICAL, CHEMICAL AND SENSORY PROPERTIES OF CONDENSED YOGHURT

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ABSTRACT: Our main goal in this project is to study the causes and finding solutions for both the hydrolytic and the oxidative rancidity that can be produced during condensed yoghurt production. The re pasteurization of the pasteurized milk and the addition of different types of antioxidants (ascorbic acid and propyl gallate) were used to achieve this goal. Chemical, physical, microbial and sensory tests were done to evaluate the product. It was found that there were significant differences between the different treatments and the control regarding the peroxide value. This means that the addition of both types of antioxidants have a positive effect in decreasing the rancidity value. However, it was found that there were some samples have hydrolytic rancidity flavour without any type of oxidative rancidity (low peroxide value). To overcome this problem the re pasteurization step was used to destroy all the vegetative form of microbes. It was found that this treatment was very useful in controlling the rancidity flavor according to the sensory evaluation of the condensed yoghurt products for several batches. The best condensed yoghurt which contains 0.25% ascorbic acid exhibited the highest sensory properties values. Also, it has the ability in lowering the oxidative rancidity in the combination with the re pasteurization step of the pasteurized milk. This suggests that a higher quality and stable condensed yoghurt can be obtained upon using this combination. These results may help producers in selecting the best treatment methods to overcome the rancidity flavor in this type of condensed yoghurt.

Key words: Antioxidant, Re pasteurization, Condensed milk

INTRODUCTION

The dairy industry is constantly trying to obtain a competitive advantage by improving their product quality. The quality of dairy products is mainly influenced by features such as texture, shape, colour, flavour, and nutritional content. In condensed yoghurt production (Zabadi), one of the main problems that arise is the production of a rancid flavour due to the microbial and chemical changes in the cream milk fat composition. Different studies were done to study the effect of various processing treatments on the dairy products quality. Krause et al. (2008) studied the effect of refrigerated and frozen storage on butter flavour and texture. Emmons et al. (1986) studied the effect of storage time on butter flavour “Grading and peroxide value (PV)” were used to evaluate butter quality. Gandy et al. (2008) studied the effect of pasteurization temperature on the shelf-life of fluid milk and its sensory acceptability.

Actually, fats in milk are subject to two types of deterioration that affect the flavours of Zabadi: 1. Hydrolytic rancidity: In hydrolytic rancidity, fatty acids are broken off from the glycerol molecule by lipase enzymes produced by milk bacteria. The resulting free fatty acids are volatile and contribute
significantly to the flavour of the yoghurt. 2. Oxidative rancidity: Oxidative rancidity occurs when fatty acids are oxidised. In milk products it causes tallowy flavours. Oxidative rancidity of dry butterfat causes off-flavours in recombined milk. For both kind of rancidity the detection and the preventing can be done. Hydrolytic rancidity (Lipolysis) can be caused through the action of milk lipoprotein lipase. One way in which this enzyme is normally prevented from hydrolyzing milk fat is by the physical barrier of the milk fat globule membrane. Agitation that results in disruption of this membrane can result in lipolysis; this ‘induced lipolysis’ is a problem that can be caused by pumping, processing and transport of milk. Microbial contamination can also cause lipolysis and, although pasteurization kills psychrotrophic bacteria, proteinases and lipases produced by them withstand normal pasteurization temp. Mainly, the agitation that happens during the milk fat separation (in the production of cream milk (50% fat) can cause a disruption of this membrane (Pennsylvania State University report, 1991). In the oxidative rancidity the role of copper in the oxidation were stressed, and the role of ascorbic acid and propylgallate in preventing lipid oxidation were studied. Casein also has a protective effect by binding Cu2+ and Fe3+ ions. Pasteurization at 80°C can reduce the pro-oxidative effects of lactoperoxidase and xanthine oxidase, but other forms of processing such as churning, encourage oxidation. Researchers have concluded that the development of objectionable flavours in pasteurized milk is generally a result of bacterial growth (Simon and Hansen, 2001), and unpleasant aromas in fluid milk are characteristic of milk spoilage (Hayes et al., 2002). However, in this research different steps were done to achieve a complete protection of a Zabadi flavour. Our main goal in this project is to study the causes and finding solutions for the rancidity that can be produced during cream milk and condensed yoghurt production (Zabadi). Different steps were done to achieve our goal. These steps are: 1. Controlling the pasteurization conditions. 2. Using natural and synthetic antioxidants (such as Vit C. and Propyl gallate) after pasteurization step. At the same time, evaluation Zabadi product after selecting the best processing conditions was done. This evaluation step was done by using different analytical and sensory tools.

MATERIAL AND METHODS

Zabadi Formulation and Production

Several steps are involved in the Zabadi (condensed yoghurt) production. These steps are:
Milk Fat (3%), Churning and fat separation, Cream milk (50% fat) addition, Pasteurization (95 °C/ 16 s), Reconstitutions and mixing with 3% milk fat to obtain milk with 10% fat, Re pasteurization (95 °C/ 16 s), Cooling to 42 °C, Inoculation with starter culture and incubation, and finally Condensed Zabadi Product (10% Fat). The following experimental design was achieved to help in solving any type of oxidative rancidity. The experimental design consisted of 6 condensed cream formulated with antioxidants produced at standard processing conditions and replicated three times as can be shown in Figure 1. The main variables which were investigated and selected for further investigations in this study are shown in Figure 1. As shown, the study was subdivided into two stages. In each stage, one treatment was selected for further investigation according to the best antioxidant properties. Yoghurt creams were immediately packed, sealed, and placed in storage at 4 °C after production.
Figure 1. The experimental design consisted of 6 condensed cream formulated with antioxidants produced at standard processing conditions and replicated three times.

### Antioxidants Addition and Fat Extraction

The antioxidants at different concentration were added directly to the pasteurized yoghurt cream samples at room temperature. The fresh pasteurized yoghurt cream (50% fat) was selected to study the effect of the antioxidants addition on the oxidative rancidity because of the highest extraction rate of the fat which will help in keep monitoring the peroxide formation. However, the pH and the peroxide value of the extracted cream fat were determined before and after the antioxidant addition. Total lipid extraction was carried out according to Folch, Lees, and Sloan (1957): 15 g of yoghurt cream was added to 200ml of chloroform : Ethanol solution (1:1, v/v), homogenized for 30 s and another 100 ml of chloroform was then added to the mixture. The mixture was again homogenized for 1 min and filtered. The filtrate was added to 100 ml of 1 mol/l KCl and was left overnight at 4°C. After phase separation, the chloroform phase was evaporated using a rotary evaporator and the lipid fraction was stored at -20±1°C.

**Peroxide determination** was done according to the following procedure:
1. Weigh 5 g of fat sample into 250ml standard Erlenmeyer flask.
2. Add 30 ml of acetic acid-chloroform (60:40) and shake vigorously.
3. Add 0.5 ml saturated Potassium iodide solution. Stopper and shake thoroughly and allow to stand in dark for exactly 2 minutes.
4. Add 30 ml distilled water and several drops of starch solution and titrate liberated iodine with standardized 0.02 N sodium thiosulphate. The end point is the transition from dark blue to clear.
5. Peroxide value is equal to (meq.100g lipid) = (titration volume-blank) * normality of thiosulphate * 100/oil weight

### Microbiological properties

Yoghurt creams were immediately packed, sealed, and placed in storage at 4 oC after production. Total aerobic plate count was determined according to standard pour plate method (Speck, 1979) on plate count agar (Standard Plate Count Agar, Conda Laboratories, S.A., Spain) incubated at 37 °C for 48 h. Bacillus sp. was identified according to their shapes on the plate count agar. The main aim behind doing the microbial examination is to find if there is a relation between the microbial growth contamination and the flavor defect on the final product.

### Sensory Evaluation of the Yoghurt Zabadi

The best yoghurt cream which contains 0.25% ascorbic acid was selected for the sensory evaluation treatment. The selection of this treatment for further study was based on its efficiency in lowering the peroxide value. Also, it is a natural antioxidant which differs from the propylgallate and finally it is formed the least amount of antioxidant that was added to the formula. It is expected that using this antioxidant at this low level will not cause any changing in the product quality. However, to achieve

<table>
<thead>
<tr>
<th>Antioxidant Concentration</th>
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<td>0.25%</td>
<td>2. Propyl gallat</td>
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<tr>
<td>0.5%</td>
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<td>200 ppm</td>
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this purpose and to find the effect of this addition on the final product the sensory evaluation was done. Addition of this antioxidant was done at the industrial level. Ascorbic acid at 0.25% was added to 25 L of Zabadi condensed cream final product (E300) after mixing the skim milk with yoghurt cream and before the filling step of the final product. The samples were collected stored at 4 C. The comparison was done between E300 treatment and the standard treatment (No antioxidant was added, E500) in terms of all the quality attributes (overall acceptance, flavour, mouthfeel, color and appearance). Sensory evaluation was conducted in the Laboratory at the Hashemite University. 6 sensory judges (3 male and 3 female) with ages between 22 and 40 were trained on descriptive analysis of yoghurt properties. Descriptive analysis panelists were consumers of yoghurt and trained for 2 hours (1-h session/day) on quality attribute profiling (overall acceptance, flavor, mouthfeel, color and appearance). The panelists were trained for evaluations of reference samples, with and without spiking with ingredients to alter sample sensory attributes. Panelists evaluated samples in standard sensory panel booths containing an attribute definition sheet. During each training session the panelists were familiarized with yoghurt quality attribute profile to be evaluated using a 9 point scale for each quality attribute using descriptive analysis. Sensory evaluation was performed on all different types of yoghurt products. These yoghurt samples were presented to the trained sensory panel in yoghurt cans. Samples were presented to the trained panels according to a random, balanced design along with 2 unsalted crackers, a glass of room-temperature distilled water for rinsing the palate between samples, a napkin, and score sheet. Panelists evaluated samples in standard sensory panel booths containing an attribute definition sheet and pencil. The panelists for each session had no prior information about the coded test products.

**Statistical analysis**

Data were analyzed by the Analysis of Variance procedure (ANOVA) by using SAS software (version 8.1, SAS Institute, Cary, NC). Comparison between treatments was analyzed using Fishers Least Significant Difference (LSD). In the analysis of variance, alpha level of 5 % was used to test the null hypothesis.

**RESULTS AND DISCUSSION**

**Peroxide and pH values**

The results of the peroxide values of the different treatments before and after antioxidants addition can be shown in Table 1. As can be shown that there were significant differences between the different treatments and the control regarding the peroxide value. This means that the addition of both types of antioxidants have a positive effect in decreasing the rancidity value. However, as can be shown that this difference is so small. It was found that during the study there were some samples have rancidity flavour without any type of oxidative rancidity (low peroxide value). Thus, it was expected that the hydrolytic rancidity could be the main reason. It was found that the raw pasteurized milk and the final Zabadi product contain uncountable Bacillus species which should not be present. This is a good indicator about the presence of the spores which are highly resisted the pasteurization treatment. However, to overcome this problem a re pasteurization treatment of the milk is so useful. The re pasteurization step have the capability to destroy all the vegetative form of Bacillus species that were formed from the spores after the first pasteurization treatment. It was found that this treatment was very useful in controlling the rancidity flavor according to the sensory evaluation of the Zabadi products for several batches. The results of the pH values of the different treatments before and after antioxidants addition can be shown in Table 1. As can be shown that there were significant differences between the different treatments and the control regarding the pH values.

**Table 1. Peroxide and pH values of the different treatments before and after the antioxidants addition.**
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<table>
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<tr>
<th>Treatments</th>
<th>Peroxide value</th>
<th>pH values</th>
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</thead>
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<td>Control (without antioxidant)</td>
<td>1.0^A ± 0.003</td>
<td>6.87^A ± 0.03</td>
</tr>
<tr>
<td>0.25% Ascorbic acid</td>
<td>0.5^B ± 0.006</td>
<td>6.80^A ± 0.04</td>
</tr>
<tr>
<td>0.5% Ascorbic acid</td>
<td>0.4^B ± 0.005</td>
<td>6.77^A ± 0.03</td>
</tr>
<tr>
<td>100 ppm Propyl gallate</td>
<td>0.5^B ± 0.004</td>
<td>6.80^A ± 0.04</td>
</tr>
<tr>
<td>200 ppm Propyl gallate</td>
<td>0.4^B ± 0.005</td>
<td>6.78^A ± 0.02</td>
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Sensory evaluation Yoghurt Cream in the presence of antioxidant

The results of the panel sensory evaluation of the condensed yoghurt produced at different stages of productions are shown in Figures 2. All the treatments exhibited sensory properties values (overall, mouthfeel, flavor, appearance and color) that were not significantly different between E300 (with antioxidant) and E500 (without antioxidant). However, E500 had a higher flavor and mouthfeel score than that of the E300, but the score was not significantly. The E300 had multiple consumer comments about the acid/sour flavors. These results suggest that the addition of 0.25% ascorbic acid as an antioxidant has not any adverse effect on the sensory properties on the other hand it has the ability in lowering the oxidative rancidity effect in the combination with the re pasteurization step of the pasteurized milk. This suggests that higher quality and stable condensed yoghurt cream could be obtained upon using these combinations.

Figure 2. Sensory properties of two types of condensed yoghurt produced by using 0.25% Ascorbic acid (E300) and without (E500, control)

The final product of the condensed yoghurt cream produced by the two different treatments can be shown in Figure 3.
Figure 3. The condensed Yoghurt (Zabadi) final product produced by addition of 0.25% ascorbic acid (E300) and without (E500).

CONCLUSIONS
1. Our goals in this project were achieved by overcoming any types of possible rancidity through the addition of the suitable antioxidants and the re pasteurization of the milk without any adverse effect on the final product.
2. The Sensory evaluation of the product before and after the antioxidants addition of the Zabadi proves that the addition of ascorbic acid at 0.25% to the final product did not cause any significant effect.
3. Determining the main factors that influence the rancidity were achieved which will help the producers to control these factors during processing and produce a stable product.

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REFERENCES