Full Length Research Paper

Effect of milk and Xanthan as egg replacement on the chemical and microbial safety properties of Mayonnaise

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The milk along with Xanthan at levels 0, 1.9, 3.9, 5.9, 9.9 and 0.1 and 0.2 % (No 1 (control) to 11 respectively) as egg replacement were used for preparation of mayonnaise. Chemical composition, acidity and pH of samples over a storage period of 3 months were investigated. Also microbial safety of mayonnaise discussed visionary. It was found that pH of samples were in the range of 3.8 to 3.98 and mean acidity were 0.71. Statistical study reveals that effect of treatment on pH of all the mayonnaise samples was significant (p<0.05) during the storage period where interplay the treatment and time on pH of all the mayonnaise samples were not significant (p>0.05). This point expressed that replacing the egg with milk caused the decrease pH of mayonnaise samples due to higher initial pH of egg in comparison to milk but during storage period was constant. To make mayonnaise microbiologically safe, it is recommended the use of vinegar as an acidulate, a pH of 4.10 or less and the product should be held in a warm place for several hours or preferably for several days before consumption. From these recommendations, it can easily be seen that to make safe mayonnaise prepared with vinegar, the pH of mayonnaise should be equal to or less than 4.10 or 4.00 (called the safe pH value) which in our study also was 3.98. In general, the death rate of the salmonellas reflected the acetic acid content rather than the pH of a mayonnaise.

Key words: Mayonnaise, Xanthan, milk, acetic acid, salmonella.

INTRODUCTION

Mayonnaise is typical oil in water emulsion prepared from vegetable oil, egg yolk, vinegar, sugar, salt, mustard and a variety of food additives (Juszczak et al. 2003). Among its ingredients, egg yolk is most critical in term of stability of the mayonnaise. Egg is considered a high profile ingredient because of its high nutritional value and multifunctional properties, including emulsification, coagulation, foaming, and flavor product (Narsimhan and Wang 2008). Cholesterol is present in the egg yolk in quantities varying from 180 to 250 mg, depending on hen genotype (Stadelman and Cotterill, 1995). The desire to replace eggs in food systems was brought about by a multitude of concerns from consumers, and processors desired to have low cholesterol and safe (without salmonella) foods (Liu et al. 2007). So there have been done several studies on removing or decreasing egg in mayonnaise (Takeda et al. 2001; Rir et al. 1994).

The emergence of Salmonella enteritidis as a major cause of human salmonellosis led to detailed studies of eggs (Humphrey, 1994) and egg products, especially mayonnaise (Radford and Board, 1993), because of their role in the transmission of this pathogen from laying hens to humans (Dick, 1993). Attention has been given mainly to mayonnaise made with unpasteurized eggs (Radford and Board, 1993). As an oil-in-water emulsion, the chemical composition of the aqueous phase will determine the fate of contaminants in mayonnaise (Tuynenburg Muys, 1971). Until recently, the literature fostered the view that acidity was the major antimicrobial feature of this phase, particularly when acetic acid was the acidulate (Smittle, 1977). Indeed, it has been recommended that the content of this acid in mayonnaise should be 0.29- 0.50% of the total product (Anon., 1975). the pH of mayonnaise, especially when poised at 4.0 with acetic acid, is a major impediment to microbial spoilage (Smittle, 1977; Radford and Board, 1993). Several
investigations have shown that these two factors lead to the death of salmonellas in mayonnaise stored at ambient temperatures (e.g. Perales and Garcia, 1990; Lock and Board, 1996). The rate of auto sterilization of mayonnaise inoculated deliberately with salmonellas is influenced, however, by factors other than pH per se. Recent observations have revealed that various ingredients as well as storage temperature enhance or ameliorate the antimicrobial action of acetic acid. Thus, Perales and Garcia (1990) found that the rate of die-off of salmonellas in mayonnaise (auto sterilization in the parlance of Leistner, 1995) stored at 24 or 34 °C was faster than that at 4 °C - a situation confirmed by Lock and Board (1995); the actual rate of death at all three temperatures being accelerated if the acidity of the commodity was increased with acetic or, to a lesser extent, citric acid.

The cardinal role of pH per se in the decontamination of commercial mayonnaise infected deliberately with salmonellas was noted by Lock and Board (1994). The inimical properties of home-made mayonnaise were lessened when supplemented with NaCl and acidulated with acetic acid (Radford and Board, 1995). In contrast, mayonnaise made with extra virgin olive oil was more bactericidal than that with blended olive or sunflower oils (Radford et al., 1991). Radford (1994) noted that mustard or a fresh extract of garlic increased the rate of die-off of salmonellas in mayonnaise. This communication presents additional information on the influence of various vegetable oils and varieties of vinegars on the antimicrobial properties of mayonnaise. These observations indicate that the formulation of recipes for mayonnaise made with unpasteurized eggs ought to pay heed to the concepts of hurdle technology (Leistner, 1995). By so doing, the selection of appropriate ingredients and storage temperature could maximize auto sterilization of this commodity.

Therefore, the present study was conducted to prepare low cholesterol and microbiologically safe mayonnaise by application a desirable combination of whole milk, Xanthan Gum, and egg, investigating the changes of acidity and pH levels during storage period.

MATERIALS AND METHODS

Mayonnaise Preparation

Two egg replacers (Xanthan gum and pasteurized, homogenized low fat milk mixture) were used in this study. According to the previous work, substitution of egg with milk and Xanthan mixtures carried out and best formulation selected and done in this study. Substitution of egg with milk and Xanthan mixtures from level of 0 -100% and 0-0.2% was selected respectively (Nikzade, 2012). Reduced egg mayonnaise samples (11 samples) were prepared according to the following procedure and by using ingredients written in Table 1. To prepare different mayonnaises, first powder ingredients (salt, sugar, Xanthan, mustard, carboxymethyl cellulose, sorbate, and benzoate) were mixed, vegetable oil was added slowly and for 5 min mixed. Vinegar gently was poured and mixed and the rest of oil was added slowly into the aqueous phase (Nikzade, 2012). Then this pre emulsion was homogenized with the stirrer operating. To compare different properties, 1 control samples (one industrial sample) were also prepared with the formulations shown in table 1. Each formulation was prepared one time and each experiment was performed in 2 replicates. After preparation, the samples kept in refrigerator (4-5 °C) until the analysis (Liu et al., 2007).

Compositional analysis

Compositional properties of milk analysed for protein, TS and acidity (Lactic acid) (AOAC, 1990), fat by the Gerber method (Rashid and Miyamoto, 2005) and pH (using pH meter 766 knick, Germany) were measured according to Bradley et al (Bradley et al, 1992). Also for Mayonnaise samples, protein, fat, ash, pH, moisture and acidity content (Acetic acid) were measured (AOAC, 1990). All the experiments were carried in two replicates.
Table 2. Compositional properties of milk used in Mayonnaise formulation

<table>
<thead>
<tr>
<th>Properties</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Total solid (%)</th>
<th>Acidity (lactic acid)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>3± 0.004</td>
<td>0.023</td>
<td>8.03±0.047</td>
<td>0.14±0</td>
<td>6.6±0.0047</td>
</tr>
</tbody>
</table>

Table 3. Compositional properties of Mayonnaise samples

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Control</td>
<td>6.63±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.93±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.74±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>6.62±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.71±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.77±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.34±0.004&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>66.46±0.056&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.55±0.008&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.78±0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34±0.004&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>66.36±0.05&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.36±0.004&lt;sup&gt;d&lt;/sup&gt;</td>
<td>26.48±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.33±0.004&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>66.23±0.05&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.19±0.004&lt;sup&gt;1&lt;/sup&gt;</td>
<td>26.89±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33±0.004&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>66.03 ± 0.05&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.01±0.008&lt;sup&gt;1&lt;/sup&gt;</td>
<td>26.55±0.05&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.32±0.004&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>66.66±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.71±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.55±0.05&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.35±0.009&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>66.51±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.55±0.005&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.6±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.34±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>66.48±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.37±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.61±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.34±0.004&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>66.29 ± 0.01&lt;sup&gt;et&lt;/sup&gt;</td>
<td>1.19±0.009&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.6±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.33±0.004&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>66.03±0.05&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.01±0.008&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25.63±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33±0.004&lt;sup&gt;cde&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Statistical analysis

The results in this study were evaluated by the completely randomised design using the software SPSS 16. The significant differences (P< 0.05) between the treatments were evaluated by the Duncan multiple range test procedure. All experimental determinations were assayed in triplicate.

RESULTS AND DISCUSSION

Chemical analysis

The compositional analysis of pasteurized, homogenized low fat milk and Mayonnaise prepared in this study given in Tables 2 and 3, respectively.

Changes in pH and Acidity during storage period

The pH values of mayonnaise samples recorded over a storage period of 3 months have been presented in figure 1. It was found that pH of freshly prepared mayonnaise samples were in the range of 3.8 to 3.98, indicating the mayonnaise to be acidic in nature. Statistical study reveals that effect of treatment on pH of all the mayonnaise samples was significant (p<0.05) during the storage period whereas interplay the treatment and time on pH of all the mayonnaise samples were not significant (p>0.05). This point expressed that replacing the egg with milk caused the decrease pH of mayonnaise samples due to higher initial pH of egg in comparison to milk (Egan et al. 1981), but during storage period was constant. The pH of all samples was found to be 3.98 or lower, which supposedly prevented the growth of food spoilage microorganisms. The safety of mayonnaise can be assessed by five microbiological safety criteria (Dodson et al. 1996). Smittle (1977) suggested that to maintain a Salmonella free commercial mayonnaise, the pH should be 4.10 or less. To make mayonnaise microbiologically safe, Radford and Board (1993) recommended the use of vinegar as an acidulant, a pH of 4.10 or less and a holding time of 24 h at 18±20°C before refrigeration. Perales and Garcia (1990) also recommended that the pH of mayonnaise should be between 4.0 and 3.60 and the product should be held in a warm place for several hours or preferably for several days before consumption. From these recommendations, it can easily be seen that to make safe mayonnaise prepared with vinegar, the pH of mayonnaise should be equal to or less than 4.10 or 4.00 (called the safe pH value) which in our study also was 3.98. In general, the rate of death of the salmonellas reflected the acetic acid content rather than the pH of a mayonnaise (Lock and Board, 1995). Herald et al. (2009) reported the pH of mayonnaise samples replaced by starch between 4.17-3.04 that the lowest pH recorded for the sample replaced with 100% starch. Also Laca et al. (2010) reported the pH of low cholesterol mayonnaise, 3.78, that proximate to our study.

In this study, in terms of acidity, significant differences between treatments were not observed (p<0.05). Mean acidity of the all samples were 0.71 (acetic acid) (figure 2). Radford et al. (1991) demonstrated that the type of oil used to prepare mayonnaise also affects the rate of auto sterilization. In their study, extra virgin olive oil, due most probably to its content of phenolics (Dallyn, 1994), caused the most rapid die-off of salmonella in mayonnaise stored at room temperature. Also Radford (1994) showed that the inclusion of mustard or a fresh extract of garlic enhanced the bactericidal potential of mayonnaise. Although official recommendations specify
preferable levels of acetic acid *per se* for controlling spoilage of mayonnaise (Anon., 1975), the production of this commodity in the kitchen of a home or an institution will entail the use of either a proprietary brand of vinegar having an unspecified content of acetic acid or, to reduce tartness, lemon juice. It has been established that the latter is less effective than vinegar for killing salmonellas in mayonnaise stored at room temperature (Perales and Garcia, 1990).

Dodson et al. (1996) used these criteria for assessing ten home-made mayonnaise recipes and found that only one recipe stood out as being very safe by the five criteria. This implies that some recipes are intrinsically safe while others are not. If an unsafe recipe is used with raw eggs to prepare mayonnaise, there is a very high risk of food poisoning. It may be the case that some or all outbreaks of food poisoning associated with the consumption of home-made mayonnaise may be due to...
unsafe recipes used in the preparation of mayonnaise. If intrinsically unsafe recipes can be eliminated, outbreaks of food poisoning associated with the consumption of mayonnaise may be reduced significantly or may even be eliminated. Xiong et al. (1998) investigated the relationship between the ingredients and pH of homemade mayonnaise and proposed a method for linking pH of mayonnaise to its ingredients used. On the basis of both the existing standard for pH (4.10) of mayonnaise and the worst mayonnaise recipe scenario, Xiong et al. (1998) recommended that for safe mayonnaise recipes using egg yolk, at least 20 ml white wine vinegar per fresh egg yolk should be used and the product should be held in a warm place for at least one day or preferably three days before its consumption or refrigeration. This recommendation, however, is not suitable for those recipes in which whole egg and egg white are used.

The concentration of acetic acid and the pH in most of the present formulations of mayonnaise is sufficient to inhibit growth of the majority of microorganisms and is lethal to many (Smittle, 1977). In fact, Because of acetic acid, potassium sorbate, sodium benzoate were used in the preparation of mayonnaise in this study, and pH of samples was under 4. Growth conditions for microorganisms not provided, hence, the changes in acidity during storage time were not observed. PH and acidity of all samples was in the optimal range. So, prepared mayonnaises in terms of safety were appropriate.

**CONCLUSION**

It was found that pH of mayonnaise samples were in the range of 3.8 to 3.98, indicating the mayonnaise to be acidic in nature. Statistical study reveals that effect of treatment on pH of all the mayonnaise samples was significant (p<0.05) during the storage period whereas interplay the treatment and time on pH of all the mayonnaise samples were not significant (p>0.05). By using the validated formula and a safe pH value (4.10 or 4.00) for mayonnaise, also totally replacing the egg by using milk can be reduce the risks and dangers due to the presence of cholesterol and salmonellae and other opportunistic pathogen microorganisms in Mayonnaise.

In general, the choice of vinegar influenced the rate of auto sterilization because of the amount of acetic acid included in a mayonnaise. When considered in the context of hurdle technology (Leistner, 1995), it is evident that many of the ingredients used to make mayonnaise interact with the key antimicrobial factors, pH 4.0 and the amount of undissolved acetic acid, thereby enhancing the antimicrobial potential of this commodity. In some instances, an ingredient such as salt may negate the antimicrobial action of acetic acid (Radford and Board, 1995). This evidence ought to be considered by those who offer advice to commercial caterers on the choice of recipes that will meet a consumer’s expectations—a bland mayonnaise having a low potential for the transmission of salmonellas. The proposed method is also useful for the development of safe mayonnaise recipes. In combination with nutritional analysis the method can be used to create new healthy and safe mayonnaise recipes.

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**REFERENCES**


