Effect of Trisodium Citrate Concentration and Soy Cheese on Meltability of Pizza Cheese

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EFFECT OF TRISODIUM CITRATE CONCENTRATION AND SOY CHEESE ON MELTABILITY OF PIZZA CHEESE

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The objective of this study was to determine the effect of soy cheese (tofu) and trisodium citrate (TSC) concentration on physicochemical properties of pizza cheese. The results indicated that fat and FDM contents of pizza cheese increased significantly with increased proportion of TSC, while moisture and protein decreased but not significantly (P < 0.05). On the other hand, the fat, FDM, and protein content decreased with increased proportion of tofu, while moisture increased. The melting area and melting degree indicated that melting properties of pizza cheese decreased significantly, as the concentration of TSC increased. Furthermore, increase approximately 5 and 10% tofu in the blends with 0.5% TSC or 10% tofu in the blends with 1% TSC affected notable decrease in melting properties. Among each level of tofu, oiling off area showed significant increase with increasing TSC. Increase in tofu proportion as well as different TSC concentration resulted in decrease in free oil. The control cheese with 1% TSC (CC2C) had maximum oiling off, and with increase in tofu proportion there was a corresponding reduction (P < 0.05) in the oiling off area. It was observed that increase approximately 10% tofu in the blends with 0.5% TSC cause marked reduction in oiling off area by approximately 47%. There was rapid increase in oiling off area during the early stage of cooking. According to observation, free oil formation of cheese with 1% TSC (C2C) was higher than that of the cheese with 0.5% TSC (C1C), especially during 2.5 to 10 min (P > 0.05). Oiling off area of treatments decreased independently of trisodium citrate prolong cooking as the proportion of tofu increased.

Keywords: Pizza cheese, Imitation cheese, Analog cheese, Tofu, Melting properties, Oiling off, Image processing.

INTRODUCTION

Cheese analogues are processed cheese substitutes solely made on the basis of dairy ingredients, or products in which dairy components are partly or entirely replaced by non-dairy ingredients, mainly of vegetable origin.\[1\] Cheese analogues are cost-effective to produce due to the incorporation of cheaper ingredients and the simple manufacturing technology,\[2\] while nutritional demands by the consumer and demands from the industry regarding further processing of cheese analogues can be more easily fulfilled than in conventional processed cheese.\[3\] On the other hand, Global production and consumption of...
pizza cheese has experienced unprecedented growth during the last two decades as a result of gains in popularity of pizza topping and related foods.\textsuperscript{14,51} Consumption of pizza cheese also has greatly increased in Iran. Considering the ever increasing popularity of pizza among children and teenagers in Iran, pizza cheese consumption is expected to grow.\textsuperscript{6} Thus, the most common types of cheese analogues are substitutes for pizza cheese.

One of the ingredients that very common in pizza cheese analogue is soybean product. Soy beans have been consumed for centuries and use in the form of soy flours and similar fractions in Asia, approximately 90% of soy bean curd or tofu.\textsuperscript{7} Soy protein is unique among plant proteins owing to its relatively high biological value, essential amino acid content and economic advantage. Studies have been undertaken on the effects of replacing casein in ACPs by various types of vegetable proteins, e.g., soybean, peanut, pea protein or wheat protein.\textsuperscript{4} These proteins gave varying results, depending on the ingredient preparation (e.g., soy flour or soy isolate, pH, fat content) and the type and level of other ingredients, e.g., hydrocolloids.\textsuperscript{4} However, the use of these protein substitutes has been found to give ACPs, which have a quality inferior to that made using casein only. Common defects include lack of elasticity, lower hardness, an adhesive/sticky body, impaired flow and stretchability and/or poor flavor.\textsuperscript{4} In addition, Ahmed et al.\textsuperscript{8} investigated the feasibility of using groundnut or soya protein as a partial replacement for casein in the manufacture of imitation cheeses. The cheeses became softer and less rubbery with increasing substitution of plant proteins.

Among the most important ingredients used in processed cheese manufacture are emulsifying salts.\textsuperscript{3} The effects, which are caused by the addition of the emulsifying salts, are calcium masking (or sequestration), pH increase and buffering, stabilization of the oil-in-water emulsion, and structure formation.\textsuperscript{9,10} During cooking, emulsifying salts help to change some of the casein from an insoluble form to a soluble form, where they can emulsify the fat. It is well known that soluble casein, such as sodium caseinate, is an excellent emulsifier because of its separate hydrophobic and hydrophilic regions on the casein molecules. Finally, addition of the salts results in a smooth and homogeneous processed cheese mass. Trisodium citrate is a common emulsifying salt, which is used extensively in the manufacture of processed cheese. Gupta et al.\textsuperscript{11} reported that the use of different types and emulsifying salt concentrations resulted in process cheese that had a wide range of pH values and degrees of hardness and meltability. However, other studies\textsuperscript{9,12} have not reported similar trends for functional properties of individual ES compared with those reported by Gupta et al.,\textsuperscript{11} probably because of differences in the experimental cheese-making conditions. Shirashoji et al.\textsuperscript{13} reported that meltability of fat droplet decreased with increasing concentration of trisodium citrate.

As pizza cheese usually consumed in the melted state, the heat-induced functional properties of pizza cheese such as melting properties (melting area and melting degree) and oiling off are essential determinants of the quality and acceptability of pizza cheese.\textsuperscript{4} Meltability is one of the most important functional properties, especially in application of cheese as topping or ingredient for some prepared consumer foods. Only after melting of cheese can some desirable appearance and properties of food product be seen e.g. fused and bright surface, stretchability, and elasticity of cheese expected on pizzas.\textsuperscript{14} Meltability of cheese reflects the ability of cheese particles to flow past one another when heated.\textsuperscript{15} Meltability is governed by protein-to-protein interactions that bind the paracaseins fibers together. Very strong interactions restrict flow became tough whereas very weak interaction may cause flow to be excessive and soupy appearance.\textsuperscript{16}
Oiling off is caused by the release of free oil from the body of melted cheese. During melting, fat globules are gradually transformed from predominantly solid in nature to completely liquid. Concurrently, the volume occupied by fat globules increases through thermal expansion, which causes the liquid globules contained within the channels to pack together more closely and coalesce as pools. Pools of liquid fat then flow and merge between collapsing and sliding layers of paracasein fibers as the fibers dissociate. A portion of the pooled liquid fat finds its way to the cheese surface, and it is expressed as free oil. Free oil formation, therefore, is governed by two critical factors during melting: (1) the coalescence of fat globules within channels to form pools of liquid fat; (2) the dissociation of paracasein fibers, which causes them to collapse and flow, thereby enabling pools of liquid oil trapped within channels to flow and merge with one another into even larger pools. Moderate release of free oil contributes to desirable melting characteristics by creating a hydrophobic film on the cheese surface during baking, giving the surface a desirable sheen and, more importantly, slowing down evaporative loss of moisture. Hence, the purpose of this research was to determine the effect of different concentrations of tofu and trisodium citrate on physicochemical properties on pizza analog cheese.

MATERIALS AND METHODS

Preparation of Soy Milk

The soy flour used in this study was obtained from Toos Soya Company, Mashhaad, Iran. One part flour was mixed with seven parts water with constant stirring for 10 min at 80°C. Antifoam spray was used to control foaming. Soy milk was extracted from the slurry after centrifugation (1200 × g) for 5 min. A single batch of soymilk was used for the preparation of tofu. This was done in order to avoid any changes in the soymilk composition.

Preparation of Tofu

Soy milk was heated to a temperature of 80°C under stirring. The time period of heating, and stirring speed was kept constant for tofu preparation. Tofu was prepared by coagulating the soymilk using calcium chloride at a concentration of 0.4% based on the amount of soymilk used. This coagulant concentration was chosen based on coagulant concentrations carried out prior to this study. Calcium chloride was dissolved completely in 20 ml of cold water and was used immediately. The hot soymilk and coagulant solution were poured simultaneously into steel container ensuring good mixing without stirring. The soymilk-coagulant suspensions were allowed to stand undisturbed for a period of 25 min to ensure that coagulation occurred. The curds thus formed were broken thoroughly and transformed into a specially designed mould (10 × 10 × 8 cm) lined with cheesecloth. The mould had perforations on sides and bottom. The whey was drained off naturally for 15 min and the cured was pressed. Next, tofu was transferred into plastic bag and stored in a refrigerator until further analysis.

Preparation of Samples

Natural (based) cheese curd and cream was obtained from a Razavi Dairy Company, Mashhad, Iran. The natural cheese was grind, stored at −10°C and was transferred to a 5°C
refrigerator one day before mixing. For pizza production, tofu curd was mixed in different proportions (0:100, 5:95, and 10:90) with natural cheese. As emulsifying salt, trisodium citrate were dissolved in water (40°C) depending on the treatment (0.5 and 1%) and added to the dry ingredient blend followed by cooking. The temperature and time (the time for which the processed cheeses were held after they placed in cooker) of cook water were approximately 80°C and 40 min, respectively. All treatments were manufactured in 1 kg batches using a single screw pilot-scale cooker blender to achieve a homogeneous paste. The cooked processed cheeses were filled hot in 1 kg boxes and transferred to the cold room (4°C) after 15 min. All the cooked pizza cheeses were wrapped and stored at 4°C until analysis. The blocks of cheese was stored at −10°C and was transferred to a refrigerator maintained at 5°C one day before slicing. Samples were cut into 5 mm thick slices using slicing machine (NOWA, Italy) and then shaped to round pieces with 17 diameters using steel mould. The slices were wrapped and stored at 4°C.

**Chemical Analysis**

The fat content of cream, soy milk, tofu, base cheese, and pizza cheese were determined by the Gerber method. Total protein content of pizza cheese samples were determined by the Kjeldahl method. Soy milk total solids was determined by drying 8 to 11 g of milk at 100°C for 4 h. Soy, base, and pizza cheeses were analyzed for moisture content by the oven drying method. Fat in dry matter (FDM) was calculated as follows:

\[
FDM = \frac{\text{% fat} \times 100}{(100 - \text{% moisture})}
\]

**Image Analysis**

A Computer Vision System (CVS) was developed for determining projected area (mm²) of the samples. The system consists of a digital camera (Canon A550, Malaysia), an image-capturing box and image analysis software (Clemex Vision Professional, PE4, Canada). A sample holder was placed at the bottom of the box and it was covered with white translucent material. Two fluorescent lamps (Farhad lightening 10W, 0.09A, Iran) were placed under the sample holder to cause the shadows, and the camera was located 15 cm above it to capture standard image from samples with as good resolution as possible. The settings were kept consistent during the entire experiment period. The whole system was switched on 5 min before tests. The images captured were saved in RGB format for melting and oiling off analyses.

**Determination of Melting Properties**

The melting properties of cheese were described by the melting area and the melting degree of cheese. For determining these properties, triplicate samples of cheese (399.93 ± 8.86 mm area, 5 ± 0.45 mm thickness and 2.08 ± 0.08 g weight) were placed on the center of piece of filter paper (No. 10311612, Whatman, UK), which set on glass Petri dish with its cover (diameter = 15 cm, height = 3 cm, and mass = 105.11 ± 5.79 g). The samples were immediately transferred to an air convective oven at 90°C. After 15 min, cheese melts where removed from the oven and cooled at room temperature for 5 min.

Melting area (MA) of cheese was represented by the difference between cheese area after cooking and before cooking:
where $A$ was the cheese area ($mm^2$) after cooking time and $A_0$ was initial cheese area ($mm^2$).

Melting degree was calculated as the ratio of area after and before cooking:

$$MD = \frac{A}{A_0} \times 100,$$

where $MD$, $A$, and $A_0$ were the melting degree (%), cheese area ($mm^2$) after and before cooking time, respectively.

**Oiling Off**

The method of Breene et al.\[20\] was applied to determine the oiling off property of cheese with some modifications. A cheese disc ($399.93 \pm 8.86$ mm area, $5 \pm 0.45$ mm thickness and $2.08 \pm 0.08$ g weight) was placed on the center of piece of filter paper (No. 10311612, Whatman, UK), which set on glass Petri dish with its cover (diameter = 15 cm, height = 3 cm, and mass = 105.11 \pm 5.79 g). The sample was immediately transferred to an air convective oven at 90°C. Free oil from the melted disc was absorbed into the filter paper and formed an oil ring. After 15 min, melted cheese removed from the oven and cooled at room temperature for 5 min. Note that melting properties and fat ring test carried out simultaneously. Oiling off area ($OA$) of cheese was corresponded to the difference between oiling off area after cooking time and that before cooking:

$$OA = A_t - A_0,$$

where $A_0$ is initial oiling off area ($mm^2$), which is zero and $A_t$ is the oiling off area ($mm^2$) after cooking time. Thus, oiling off area was equal to $A_t$.

**Oiling Off Prolong Cooking**

Slices of pizza cheese ($393.34 \pm 7.59$ mm diameter, $5 \pm 0.53$ mm thickness and $2.08 \pm 0.11$ g weight) were placed on the center of piece of filter paper (No. 10311612, Whatman, UK), which set on glass Petri dish with its cover (diameter = 15 cm, height = 3 cm, and mass = 105.11 \pm 5.79 g) and cooked at 120°C for up to 10 min in air convective oven. The Petri dishes were removed from the oven at periodic time intervals during cooking for image capturing.

**Statistical Analyses**

All experiments were replicated 3 times in completely randomized design. Analysis of variance (ANOVA) was carried out using MSTATC software (version 1.42). Duncan’s multiple rang test was used as a guide for comparison of the means. The level of significance was determined at $P < 0.05$. 

\[180x660\]
RESULTS AND DISCUSSION

Chemical Composition

The compositions of the samples are summarized in Table 1. As the concentration of TSC increased, a tendency towards higher (P < 0.05) overall fat and FDM contents was seen consistently on each levels of tofu. This could be due to stabilization of oil in water emulsions. Results obtained in this experiment suggest that moisture and protein decreased with increased concentration of TSC. In addition, fat, FDM and protein contents decreased as the tofu substitution increased. This effect concurs with the result of the others,[21,22] who reported that increase soy protein in cheese could be the occasion of fat and protein reduction. On the other hand, the moisture content increased, as the proportion of tofu increased. This may be a result of hydrophilic nature of soy proteins.[21–23]

Melting Properties

The results showing the effect of tofu and TSC on melting properties of cheese are presented in Tables 2 and 3. The melting area of cheese was found to lie between 7.07 to 71.77 mm². It was found that CC1C and IC2T2C had the greatest and lowest value of melting area, respectively. Among the level of tofu, melting area decreased significantly with increasing TSC. ANOVA illustrate that the melt area of cheese samples did decrease with increased proportion of soy cheese, which the trend was significant for IC1T1C and

<table>
<thead>
<tr>
<th>Component</th>
<th>TSC concentration</th>
<th>Proportion of tofu in blend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>0.5%</td>
<td>51.10 ± 3.20 Aa</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>48.77 ± 6.44 Aa</td>
</tr>
<tr>
<td>Fat, %</td>
<td>0.5%</td>
<td>19.30 ± 1.32 Ab</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>24.50 ± 2.57 Aa</td>
</tr>
<tr>
<td>FDM, %</td>
<td>0.5%</td>
<td>39.58 ± 3.88 Ab</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>47.94 ± 2.55 Aa</td>
</tr>
<tr>
<td>Protein, %</td>
<td>0.5%</td>
<td>24.82 ± 2.93 Aa</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>21.36 ± 1.71 Aa</td>
</tr>
</tbody>
</table>

For each test, the means followed by the same capital letter is in a row and same lower case letter in a column, do not differ statistically at 5% probability through the Duncan’s Multiple Rang Test.

<table>
<thead>
<tr>
<th>Tofu (%)</th>
<th>TSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>51.77 Aa</td>
</tr>
<tr>
<td>5</td>
<td>62.17 Ab</td>
</tr>
<tr>
<td>10</td>
<td>58.57 Ab</td>
</tr>
</tbody>
</table>

For each test, the means followed by the same capital letter is in a row and same lower case letter in a column, do not differ statistically at 5% probability through the Duncan’s Multiple Rang Test.
Table 3 Average values of melting degree (%) of imitation cheese made with different levels of tofu and trisodium citrate.

<table>
<thead>
<tr>
<th>Tofu (%)</th>
<th>TSC (%)</th>
<th>0.5</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>118.4 Aa</td>
<td>104.5 Ba</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>115.6 Ab</td>
<td>103.1 Bab</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>114.8 Ab</td>
<td>101.7 Ba</td>
</tr>
</tbody>
</table>

For each test, the means followed by the same capital letter is in a row and same lower case letter in a column, do not differ statistically at 5% probability through the Duncan’s Multiple Rang Test.

IC2T2C as compared to CC1C and CC2C, respectively. Conversely, the melting degree (%) of cheese determined at different levels of soy cheese varied from 101.7 to 118.4%. Compared to other cheeses, the control cheese had the highest value of melting degree. The melting degree represents significant reduction as the concentration of TSC increased. Furthermore, melting degree decreased with increased proportion of tofu, and the trend was significant for IC1T1C and IC2T2C as compared to CC1C and CC2C, respectively.

Similar trends were observed for melting area and melting degree, which determine melting property of cheese. Shirashoji et al.\cite{13} showed that loss tangent parameter (from small amplitude oscillatory rheology), extent of flow (derived from the University of Wisconsin Meltprofiler), and melting area (from the Schreiber test) all indicated that the meltability of process cheese decreased with increased concentration of TSC. Gupta et al.\cite{11} reported that the use of different types and concentrations of ES resulted in process cheese that had a wide range of pH values and degrees of hardness and meltability. They reported that meltability of process cheese decreased as the concentration of TSC increased from 1.4–3.2%.

Lee and Marshall\cite{24} reported that adding soy protein to process cheese was destructive to microstructure and texture, which heat-denatured soy protein was more effective. Soy protein caused reduction in emulsifying capability of casein, increasing coarse (large hole), and inhomogeneous structure in process cheese. Thus, soy proteins behave as interrupted composite structure, reduce the ability of cheese particles to flow past one another and cause poor flowability.

According to Fox et al.\cite{4}, replacing casein in analogue cheese products by various types of vegetable proteins (e.g., soybean) was found to give ACPs which had a quality inferior to that made using casein only. Common defects include lack of elasticity, lower hardness, an adhesive/sticky body, impaired flow and stretchability and/or poor flavor. Several studies on process or analog cheese reported that meltability of cheese decreased as the proportion of soy solids increased.\cite{22,25}

**Oiling Off**

Data on oiling off area of the various cheeses are summarized in Table 4. The oiling off area of melted cheese ranged from 988 to 7100 mm$^2$, the highest and the lowest values are for CC2C and IC2T1C, respectively. Results indicated that there was marked increase as the concentration of TSC increased. On the other hand, increase in tofu proportion as well as different TSC concentrations resulted in decrease in free oil. The CC2C had maximum oiling off between C2Cs, and with the increase tofu proportion there was a
Table 4  Average values of oiling off area (mm$^2$) of imitation cheese made with different levels of tofu and trisodium citrate.

<table>
<thead>
<tr>
<th>Tofu (%)</th>
<th>TSC (%)</th>
<th>0.5</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>1867 Ba</td>
<td>7100 Aa</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>1433 Bab</td>
<td>5533 Ab</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>988 Bb</td>
<td>3733 Ac</td>
</tr>
</tbody>
</table>

For each test, the means followed by the same capital letter is in a row and same lower case letter in a column, do not differ statistically at 5% probability through the Duncan’s Multiple Range Test.

corresponding reduction (P < 0.05) in the oiling off area. It was observed that an increase of approximately 5 and 10% of tofu in the blends (IC1T2C and IC2T2C as compared to CC2C) caused decrease in oiling off area by approximately 1.3 and 1.8 times, respectively. The emulsifying salts are incorporated to enhance protein hydration and the emulsification of free fat.$^{[3]}$ Fat globules that are emulsified, rather than free oil entrapped within voids in the protein matrix, have been reported to contribute to free oil formation.$^{[26]}$ Free oil formation in pizza cheese increased with increasing FDM content. Free oil increases in an exponential rather than linear manner in relation to FDM. In general, the rate of production of free oil increases very gradually with increasing FDM between about 10% and 30%, more rapidly between 30% and 40%, and extremely rapidly above 40%.$^{[16]}$ Consequently, pizza cheese with high FDM (C2C as compared to C1C) is highly prone to the production of excessive free oil on melting. On the other hand, small reductions in FDM in the range of 40–50% can achieve surprisingly large reductions in free oil formation. Hence, there is notable difference among C2Cs, which have 47.94 to 44.34% FDM, with different proportion of soy cheese, but non significant in free oil of pizza cheese by addition soy cheese with FDM varied from 39.58 to 35.70%.

Oiling Off Prolong Cooking

A plot of oiling off area versus cooking time at different levels of TSC and tofu is given in Fig. 1. There was rapid increase in oiling off area during the early stages of cooking, which is corroborate the enhanced of free oil formation of pizza cheese. McSweeny.$^{[16]}$ reported that free oil formation increased during melting. Wang and Sun.$^{[27]}$ reported that oiling off increase rapidly during the early stage of Cheddar and Mozzarella cooking.

From the results, free oil formation of C2Cs was higher than that of the C1Cs, especially during 2.5 to 5 min (P > 0.05). This could be as a result of higher fat content of C2Cs than C1Cs. This is in accordance with the findings of Lefevere et al.$^{[28]}$ who determined that cheese fat is a driving force for cheese oiling off. Several researchers.$^{[16,29]}$ reported that free oil increased in mozzarella cheese at higher fat or FDM levels. On the other hand, oiling off area of treatments decreased independently of TSC as the proportion of soy cheese increased. This also attributes to FDM, which decreased with increasing tofu.

As investigation of CC2C at 10 min, approximately plateau stage during the rest of heating, when free oil area fell only slightly. At higher cooking time, an intensified decreased in oiling off area after its maximum value. This may be ascribed to that free oil undergoes evaporation with extent cooking. Thus, amount of free oil reduced at the cheese
CONCLUSIONS

The study indicated that the increasing TSC and soy cheese had major effects on the composition, melting, and oiling off properties of soy pizza analog cheese. Fat and FDM contents of pizza cheese increased significantly with increased proportion of TSC, while moisture and protein decreased but not significantly (P < 0.05). On the other hand, the fat, FDM, and moisture content decreased with increased the proportion of tofu, while moisture increased. Melting properties (melting area and melting degree) decreased, oiling of and oiling off prolong cooking increased as the concentration off TSC increased. Conversely, all physical properties studied were decreased with increasing the proportion of soy cheese.

ACKNOWLEDGMENTS

The authors would like to thank Ferdowsi University of Mashhad for providing laboratory facilities. Special thanks are due to Mahdi zarei and Reza Hajmohammadi Farimani for their advice and help during the experimentation and preparation of this research work.

NOMENCLATURE

ACP Analogue cheese product
C1C Cheese with 0.5% trisodium citrate
C2C Cheese with 1% trisodium citrate
CC1C Control cheese with 0.5% trisodium citrate
CC2C Control cheese with 1% trisodium citrate
ES Emulsifying salt
IC1T1C Imitation cheese with 5% tofu and 0.5% trisodium citrate
IC2T1C  Imitation cheese with 10% tofu and 0.5% trisodium citrate
IC1T2C  Imitation cheese with 5% tofu and 1% trisodium citrate
IC2T2C  Imitation cheese with 10% tofu and 1% trisodium citrate
TSC    Trisodium citrate

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