Laboratory production of tomato juice-based beverages - Soy Milk

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ABSTRACT

Tomato and soy milk contain large amounts of phytochemical and because of their health, its use is recommended. But the off- taste of soy protein products is the main limitation to use it. A way to reduce this problem, the use of combinations of fruits and vegetables. In this study different ratio soy milk(0:20:30:40%) contain 0.5% emulsifier with mixed tomato juice (Containing extracts of purple carrots1%, vegetables 1%, red bell pepper 2%, citric acid0.03%, salt 0.5%, peppermint flavor 0.02% and collagen 2%) were combined. DPPH free radical scavenging assay of the total antioxidant values and Hedonic methods for sensory evaluation of beverages containing different proportions of soy milk Was done. Beverage showing a better average of sensory and antioxidant characteristics underwent different heat treatments (75°C- 21min, 82°C- 21min, 88°C- 21min, 88°C- 30min, 98°C- 3min and 98°C-6min ) and was exposed to microbial assay. The results showed that total antioxidant activity increased with increasing amounts of soy milk (P <0.05), but overall acceptability was reduced (P <0.05). Microbial analysis showed that only in two treatments (30min, 88°C); (6min, 98°C) a standard microbial count was obtained (p<0.05).

Key words: tomato juice, soy milk, heat treatment process, the total antioxidant activity.

Introduction

Unbalanced nutrition contributes to prevalence of disease. Thus nowadays not only nutrient supply is important but an also medical aspect of nutrients (prevention and treatment) is of concern [4]. Tomato and tomato-based products contain a large amount of phytochemicals, minerals and vitamin, and lycopene is the predominant carotenoid pigment [13]. Soybean is regarded as a functional food due to natural health ingredients. It contains significant amounts of protein, carbohydrates, fiber, vitamin and minerals and also is the most common source of dietary isoflavones. The amount of phytostrogenses found in soy protein is depended on processing technology used and the type of soy products [25,8]. In order to reduce soy off- flavor, soy was mixed with different materials [21,11,6,27]. Milk protein, soy isoflavones; hydrolyzed collagen and Xylitol are known as a nutrient effective in improving bone mineral density (BMD). Collagen is a protein useful for health of bones and joints. BMD wad increased with daily consumption of 10 grams of hydrolyzed collagen for a period of 4-24 weeks [7]. The skin impedance decreasing with age was increased in response to Ca-collage matrix leading to skin rejuvenation through smoothening and moistening of the skin [12]. Although tomato juice is an acidic product, if it is exposed to heating process typical for acidic products the growth of Bacillus coagulants will be probable. This microorganism is of soil origin and can produce spores (karim, 1370). So soy products are functional but disadvantages are related to their off-tastes. [21,11]. The aim of this study was to produce products based on tomato juice- soy milk of high antioxidant activity according to consumer preference and to investigate the effect of heating process on microbial characteristics of the acid product.

Materials And Methods

Soymilk was prepared from Max Soy Company as sterile- paced. Tomato juice was extracted from tomatoes grown in Varamin city, Extracts of red bell peppers, purple carrot and vegetables (containing
carrot, Celery, red beet, spinach, lettuce, onion, parsley and rosemary) were obtained from French Company, Diana. Collagen from Rousselot Co. (France), emulsifier from National starch Co.(U.S), citric acid and peppermint flavor from Merck and Bell Co. (Germany) were purchased.

**Chemicals:**

2,2- Diphenyl-1- picrylhydrazyl (DPPH˙) was obtained from sigma company’s and ethanol was prepared from Merck Company.

**Microbial:**

For bacteria resistant to incubation media (OSA) lactic acid bacteria (MRS agar), yeast and mold (YGC agar) obtained from Merck Co. were used.

**Preparing materials:**

7 kg tomatoes grown in Varamin prepared, washed and dried followed by separation of pale and pasted ones. Tomatoes were placed in a pot filled with water up to 25% and blanched at temperature of 92℃ for 2 minutes according to method of (Hsu, 2008 and de Man., 1990). Sample was then completely homogenized and filtered. Filtration was performed using 3- layers sterile bandages. After separating the peel, seed and a portion of pulp (until water- soluble solids reach to 7g/100g) natural tomato juice was enriched with extracts of red bell pepper (2%), peppermint flavor (0.02%), purple carrot (1%), vegetables (1%) and salt (0.5%) to improve nutritional and sensory properties followed by quantification of total antioxidant content. The obtained mixture was regarded as control and mixed with different ratios of soymilk (0:20:30:40) containing 2% collagen and 0.5% emulsifier to improve its nutritional value. The best sample in terms of sensory and antioxidant properties were then selected. Five liters of this sample was poured in test tube, heated in a water bath at laboratory scale in different temperature ranges and different times (75℃, 21min; 82℃, 21min; 88℃, 21min; 88℃, 30min). Microbial properties of the samples were then evaluated.

**Sensory analysis:**

Sensory properties including taste and flavor, color, aroma, texture and chalk flavor were evaluated by 10 trained assessors through a five- points scaling including 1- very bad 2- bad 3- intermediate 4- good 5- very good [5].

**Determining total antioxidant activity:**

**Radical scavenging activity:**

DPPH˙. The free radical scavenging capacity was done according to Rebecca et al., [30] with a little modification, in doing so Optizen 2120 spectrophotometer (South Korea) was used. Results were expressed as scavenging percent of free radicals.

**Microbial assay:**

All microbial experiments were done based on Iran National Standard No. 3414 (unanimous, 1385).

**Statistical analysis:**

This experiment was done based on simple factorial design at three replications. Statistical analysis was performed using Minitab software and through ANOVA Analyses.

**Results:**

Table 1 indicates total antioxidant activity based on average percent of scavenging DPPH˙. As it shown different ratios of milk led to significant differences and the samples containing 40% soy milk showed the highest amount of antioxidant(p<0.05).

Table 2 shows sensory analysis of soymilk at different concentrations. Except for non-acceptable materials there is a significant difference in color, beany flavor, texture, aroma, taste and overall acceptability between treatments. Sample containing 20% soymilk was given the highest score (p<0.05) after control.

Table 3 presents microbial analysis of yeast, mold, lactic acid bacteria and acid- resistant bacteria. As one can see at different heat treatments a significant difference was observed and only in two treatments (30min, 88℃); (6min, 98℃) a standard microbial count was obtained (p<0.05).

**Discussion:**

<table>
<thead>
<tr>
<th>Sample</th>
<th>(Mean ± SD) (%) Total antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soymilk 0%</td>
<td>16 / 67 ± 0 / 577</td>
</tr>
<tr>
<td>Soymilk 20%</td>
<td>29 / 00 ± 1 / 527</td>
</tr>
<tr>
<td>Soymilk 30%</td>
<td>33 / 33 ± 2 / 887</td>
</tr>
<tr>
<td>Soymilk 40%</td>
<td>56 / 67 ± 1 / 000</td>
</tr>
</tbody>
</table>

Column means with different letters are significantly different using Tukey's test (p<0.05).
As one can see from table 1 an increase in concentration of soy milk led to increase in antioxidant activity so that control sample showed the lowest antioxidant activity and sample containing 40% soy milk had the highest antioxidant activity (p<0.05). This increase can be associated with the following factors; the first factor is increase in the amount of soy milk. Soybean is a rich source of soy protein and soy isoflavones [23,13]. Isoflavones is categorized in a group of chemicals known as phytoestrogen or estrogens derived from plants [14]. Antioxidants of phenolic structure have a high efficiency due to resonance of free radical formed which leads to inhibition of oxidation (Fatemi, 1381). On the other hand sterile soy milk was added to tomato juice blanched at 92°C for 2 min. thus the kind of heating process is another factor. Studies have shown that conventional heating process (Boil the milk in the an open pan for 20-30 min) and UHT (143°F, 60 sec) increases antioxidant activity of soy milk extracted from yellow bean varieties when scavenging free radicals thus decreasing total antioxidant power [34]; i.e. by donating one hydrogen atom to free radical present in the environment, antioxidant power of antioxidant is decreased due to change into radical state however it’s activity is increased as a result of scavenging free radicals. Soy variety is the third factor. In this research yellow soy bean varieties have been used so that the amount of phytostrogen found in soy protein was depended on technology of process used as well as on soy variety. [8] UHT processing increases potential of Oxygen radical absorption of yellow bean varieties [34]. Heating process is the forth factor. It has been shown that the amount of dietary natural antioxidant is decreased during processing although recently it was demonstrated that heating process could form compounds of new antioxidant properties [24]. The reason is that isoflavones present in soy bond to glucose covalently [18]. Heating process leads to breakage of this bond, releasing glycoside compounds and consequently enhanced antioxidant activity. Based on studies efficiency of antioxidants is related to ease of H₂ separation which can be catalyzed by heat .In other words heat facilitates release of isoflavones and bonded Hydrogen thus increasing antioxidant activity. Also there is a possibility of synergism during which activity of an antioxidant is reinforced (Fatemi, 1381).Previous studies on fruit and vegetables have shown that processing increases antioxidant potential through improving antioxidant properties or formation of new compounds such as products of mailard reaction [24,22]. Improved antioxidant properties, it is expressed that, for example, a phenolic antioxidant Such as hydroquinone and peroxy radical may be combined and form hydroquinone and Semiquinon. In this way part of antioxidant consumed is reconstructed. Semiquinon radical can also be combined with a proxy radical and end oxidation process. Thus such an antioxidant influences on oxidation process in two turns (Fatemi, 1381). During mailard reaction soy milk browns under heating process. Enhancement of antioxidant activity of processed soy milk maybe due to formation of new compounds of antioxidant properties of inter- compound transition by which some compounds are transported from lower antioxidant level to higher levels. The antioxidant effect of these substances has been noted especially about linoleic acid oxidation. Also it has been found that when alanin and prolin activate in Mallard reaction the highest antioxidant effect will be obtained (Fatemi, 1381). The fifth factor is measurement method. Product and measurement methods both of them are able to effective on amount of antioxidant measurement method of antioxidant activity of DPPH• and FRAP in soy milk follows always the same trend (as traditional processing and UHT). ORAC method in UHT processing is similar to DPPH and FRAP however in traditional processing is different from the said methods. This difference can be explained by antioxidant mechanism; ORAC reaction indicates mechanism of transfer and bond of H₂ while DPPH and FRAP represent mechanism of transfer and involvement of single electrons [29]. In this study total antioxidant activity was measured by free radical scavenging method of DPPH based on revival of free radical of DPPH with one electron. DPPH is purple and shows the highest absorbance at 517 nm. When this radical is recovered from antioxidant through absorption of one atom (like H₂) its color turns into yellow and consequently its absorbance wavelength increases from 517 to 6960- 11640nm. As a result the following reaction will be happened DPPH•+RH → DPPH-H + R• [28] It can be concluded that variety of soy bean, the amount of soy bean, heating process and processing method may enhance total antioxidant activity.

**Sensory analysis:**

<table>
<thead>
<tr>
<th>Sensory Characteristic</th>
<th>Acceptability</th>
<th>Overall</th>
<th>A</th>
<th>A</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soymilk 0%</td>
<td>29 ± 0 / 286</td>
<td>70 ± 0 / 823</td>
<td>90 ± 0 / 316</td>
<td>00 ± 0 / 000</td>
<td></td>
</tr>
<tr>
<td>Soymilk 20%</td>
<td>41/6 ± 0 / 359</td>
<td>40 ± 0 / 516</td>
<td>70 ± 0 / 483</td>
<td>50 ± 0 / 707</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Mean comparison sensory characteristic beverages in different proportion of soymilk**
Table 3: Mean comparison sensory characteristic beverages in different proportion of soymilk

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color</th>
<th>Aroma</th>
<th>Taste</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soymilk 0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>00 ± 0 / 000</td>
<td>/ 60 ± 1 / 265</td>
<td>/ 70 ± 0 / 483</td>
<td>5 / 80 ± 0 / 632</td>
</tr>
<tr>
<td>Soymilk 20%</td>
<td>/ 40 ± 0 / 843</td>
<td>/ 70 ± 0 / 675</td>
<td>40 ± 0 / 699</td>
<td>/ 00 ± 0 / 667</td>
</tr>
<tr>
<td>Soymilk 30%</td>
<td>/ 10 ± 0 / 876</td>
<td>/ 265 ± 1 / 265</td>
<td>/ 80 ± 1 / 033</td>
<td>2 / 80 ± 1 / 033</td>
</tr>
<tr>
<td>Soymilk 40%</td>
<td>/ 00 ± 0 / 816</td>
<td>2 / 10 ± 1 / 101</td>
<td>/ 50 ± 0 / 972</td>
<td>/ 30 ± 0 / 675</td>
</tr>
</tbody>
</table>

Column means (SD ± n=10) with different letters are significantly different using Tukey’s test (p<0.05).

Table 2, 3 shows sensory analysis of soymilk at different concentrations. Except for non-acceptable materials there is a significant difference in color, beany flavor, texture, aroma, taste and overall acceptability between treatments. Sample containing 20% soymilk was given the highest score and samples containing 40% soy milk had the lowest mean (p<0.05) after control.

Conclusion on sensory analysis and total antioxidant activity:

Since consumption of antioxidants improves body resistance and confers anti-cancer effect, and since taste, flavor and total sensory properties are favored by people, a sample should be selected which has higher sensory scores while exhibiting a high antioxidant activity. In this regard sample containing 20% soy milk is recommended.

Microbial analysis:

Table 4: Assessment of microbial in beverages containing 20% soy milk, in the process of thermal taining 20% soy milk

<table>
<thead>
<tr>
<th>(75°С; 30)</th>
<th>(82°С; 21)</th>
<th>(88°С; 30)</th>
<th>(94°С; 3)</th>
<th>(98°С; 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 / 00 ± 1 / 004</td>
<td>100 / 00 ± 0 / 004</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
</tr>
<tr>
<td>40 / 00 ± 1 / 004</td>
<td>60 / 00 ± 1 / 004</td>
<td>30 / 00 ± 2 / 004</td>
<td>30 / 00 ± 2 / 004</td>
<td>30 / 00 ± 2 / 004</td>
</tr>
<tr>
<td>20 / 00 ± 1 / 004</td>
<td>40 / 00 ± 1 / 004</td>
<td>20 / 00 ± 1 / 004</td>
<td>20 / 00 ± 1 / 004</td>
<td>20 / 00 ± 1 / 004</td>
</tr>
<tr>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
</tr>
<tr>
<td>20 / 00 ± 1 / 004</td>
<td>30 / 00 ± 1 / 004</td>
<td>10 / 00 ± 1 / 004</td>
<td>10 / 00 ± 1 / 004</td>
<td>10 / 00 ± 1 / 004</td>
</tr>
</tbody>
</table>

Column means (SD ± n=3) with different letters are significantly different using Tukey’s test (p<0.05).

Table 5: Assessment of microbial in beverages containing 20% soy milk, in the process of thermal Containing 20% soy milk

<table>
<thead>
<tr>
<th>(75°С; 30)</th>
<th>(82°С; 21)</th>
<th>(88°С; 30)</th>
<th>(94°С; 3)</th>
<th>(98°С; 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 / 00 ± 0 / 254</td>
<td>60 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
</tr>
<tr>
<td>45 / 00 ± 0 / 254</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
<td>50 / 00 ± 1 / 004</td>
</tr>
<tr>
<td>27 / 50 ± 0 / 664</td>
<td>30 / 00 ± 1 / 004</td>
<td>30 / 00 ± 1 / 004</td>
<td>30 / 00 ± 1 / 004</td>
<td>30 / 00 ± 1 / 004</td>
</tr>
<tr>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
<td>0 / 00 ± 0 / 004</td>
</tr>
<tr>
<td>20 / 00 ± 0 / 504</td>
<td>20 / 00 ± 1 / 004</td>
<td>20 / 00 ± 1 / 004</td>
<td>20 / 00 ± 1 / 004</td>
<td>20 / 00 ± 1 / 004</td>
</tr>
</tbody>
</table>

Column means (SD ± n=3) with different letters are significantly different using Tukey’s test (p<0.05).

Tables 4 and 5 indicate that there is a significant difference in average mold count between treatments since probability value obtained from f-test is less than 0.05. Treatments of (88°С, 30min) and (98°С, 6min)
Although tomato juice is an acidic product if it is exposed to heating process typical for acidic products will be decayed due to presence of a heat-resistant bacterium known as Bacillus coagulants [9]. It is a non-pathologic, active, saprogenic and aerobic bacterium of 10 flagella per cell. This microorganism is regarded as a gram-positive bacterium however a few strains of this bacterium react negatively when coloring. Tomato juice decayed by this microorganism gave a drug, phenolic and fruit flavor accompanied by a decreasing pH from 0.3 to 0.5 (Karim, 1370). Although a heating process may at least kill vegetative bacteria cells, when there is a high count of spores a juice with a pH of 4.30 or even 4.25 can’t suppress growth of B. coagulants [31]. There are many recommendations on meeting hygienic principals, good storage and good washing of tomatoes. If these recommendations are accompanied by an appropriate thermal process, the best method for controlling the said bacteria will be obtained [20].

In this research it was attempted to use whole tomatoes in order to prevent from original infection and to apply sterile tools next to flame (like aseptic condition) in order to inhibit secondary infection. Heat resistance of Bacillus coagulants in tomato products has, been studied by several authors and F values have been determined for different products has, been studied by several authors and F values have been determined for different temperatures. This value is regarded as to be 0.7 equivalent to sterilization (F0) value [20]. D value can be influenced by pH, Brix degree and water activity. In present research citric acid was used as flavor and preservative. Results showed that citric acid had an inhibitory effect on sporogenous bacteria. Previous studies on heating process of tomato juice have recommended common boiling water processing of tomato juice. This include hot filling (90.5°C) followed by cooling in the air or hot filling, storage and cooling by water. In this research in order to retain nutritional value and to improve sensory, physical and chemical properties, pasteurization process was used. To do this a range of heating processes regarded and microbial count was assessed.

Results showed that although under common conditions similar to two heating processes namely cool-filling (94°C, 3 min) and hot-filling (88°C, 21min) a standard microbial count hasn’t been reached, a slight increase in time and temperature and observance of aseptic condition led to desirable results.

**Conclusion:**

Soymilk contains antioxidant phenolic compounds that are enhanced under heat. Results showed that total antioxidant activity was increased with ratio of sterile soy milk (p<0/05). Soy milk off-flavor enhancing by soy milk ratio decreased total acceptability of the product. Therefore sample containing 20% soy milk that had higher antioxidant activity than control and that showed better sensory properties than samples containing 30% and 40% milk was selected as the best sample.

Results obtained from microbial analysis of 20% soy milk showed that among different heating processes including 75°C-21min, 82°C-21min, 88°C-21min, 88°C-30min, 98°C-3min and 98°C-6min only under two treatments (88°C-30, 98°C – 6min) microbial count reached to standard limit regarding lactic acid bacteria, acid-resistant bacteria, yeast and mold (p<0/05).

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**References**

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