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Evaluation of the Antioxidant Activity of Massecuit III in Pasteurized Chocolate Milk Formulation

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ABSTRACT: Chocolate milk is a popular tasteful dairy product and sugar is one of the main participants in its formulation. Massecuit III is a type of brown sugar consisting of phenolic compounds and other components that might exhibit antioxidant activity and could improve and affect the nutritional properties of the food. The aim of the present study is to replace some or all of the white sugar used in the formulation of pasteurized chocolate milk with massecuit III and evaluate its possible antioxidant activity. The new formulated product might be produced less expensive with improved quality. In order to evaluate the investigation, series of testes consisting of pH measurement, invert sugar content, sulfated ash, color determination and degree of the polarization were carried out on the sample (massecuit III). Phenolic compounds were determined by HPLC application and the antioxidant activity was evaluated using DPPH method. Total phenolic compounds in massecuit III was determined to be 126.27 mg/kg. It was concluded that by increasing the massecuit III concentration, the antioxidant activity was increased while other factors namely pH, acidity and bacterial counts were in the acceptable standard range.

Keywords: *Antioxidant Activity, Massecuit III, Pasteurized Chocolate Milk.*

Introduction

Chocolate milk is one of the most popular and tasteful dairy products. It consists of milk, sugar, cocoa powder and some hydrocolloides. Sugar is one of the main participants in its formulation (Sharaf o din, 2011). Normally brown sugar has 88-93% sucrose (Payet *et al*., 2005). Yellow and brown sugars have polyphenol as the result of minimum processing (Nakaya, 2009). The presence of phenolic acid, flavonoids, phelanoeeds and other phenolic compounds might prove the presence of antioxidant activity. In fact, there is a direct relationship between the antioxidant activity and the phenolic compounds of plants extraction (Almeida *et al*., 2006). Moreover, it has been suggested in several studies that the aromatic compounds produced as the result of Millard interactions in sugar production exhibit antioxidant activity (Payet *et al*., 2005). Sugar used in the present study is massecuit III which is a brown sugar (Moatamed zadegan *et al*., 2010). Nakason *et al*. (1996) studied the antioxidant compounds extracted from kokuto (noncentrifuged sugar) and found a considerable antioxidant activity. Payet *et al*. (2006) studied the phenolic compounds in products of sugar factory and concluded that these products, particularly molasse, contain considerable quantities of natural

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antioxidants. Therefore the application of such product that contains antioxidant is suggested (Phillips, 2009) and also it should be considered that the replacement of white sugar with brown sugar, improves the nutritional properties and on the other hand, it decreases the total price of chocolate milk produced.

Materials and Methods

Massecuit III was provided by Hamedan sugar factory. 2, 2 dephenyl-1 picrylhydrazyl was purchased from Aldrich Sigma Chemical Company of Germany. Chocolate powder was obtained from Bandesdorp, Germany and Delfi in Malaysia. The stabilizer was obtained from Poly Gel, Germany and finally vanilla essence was provided by Roberte of France. Other chemicals were purchased from Merck Chemical Company, Germany. The chocolate milk was produced according to the formulation recommended by one of the leading dairy companies in Iran. The compounds used in the main formula consisted of 91.01% milk with 1.8% standard fat, 0.8% cocoa powder (a blend of 1:1 Bendesdorp of Germany and Delfi of Malaysia), 0.125%VP-388 stabilizer, 0.25% salt, 8% sugar and 0.01% vanilla essence. In this research work, white sugar was replaced by massecuit III in four different quantities (25, 50, 75 and 100%).

Series of tests consisted of the following determinations were carried out on the massecuit III sample:

- Sugar was identified by polarimetric method according to Asadi, 2007.
- Inverted sugar was determined according to Loaf Shorl method, ICUMSA Methods book (Anonymous, 2009).
- Sulphate ash along with the inorganic compounds were evaluated by heating the sample in furnace according to ICUMSA Methods book, (Anonymous, 2009).
- pH measurement was performed on the samples using pH meter, according to ICUMSA Methods book (Anonymous, 2009).
- Color of the sugar solution was determined by employing spectrophotometer according to ICUMSA Methods book, (Anonymous, 2009).
- The existence of Arsenic and Lead was determined using Atomic Absorption Spectroscopy according to ICUMSA Methods book, (Anonymous, 2009).
- Sugar moisture test was determined by Loss on Drying method according to ICUMSA Methods book, (Anonymous, 2009).
- Total polyphenolic compounds were identified using COI/T.20/Doc No 29 (Anonymous, 2009) method.
- The antioxidant activity of the sugar was assessed by using DPPH radical scavenging assay (Sakac *et al*., 2011; Molyneux, 2004).

- *Chocolate Milk Tests*

The tests regarding chocolate milk concerned with the acidity, pH and total bacterial count were carried out according to the Iran National Standard, method number 1527. The antioxidant activity was assessed by using DPPH radical scavenging assay (Sakac *et al.*, 2011; Molyneux, 2004).

Sensory properties of the chocolate milk, color, aroma, taste, texture, sweetness and acceptability was assessed by applying the hedonic 9 point scale by 8 trained sensory panelists through filling sensual assessment questionnaire. Where numbers 1 indicates the least and 9 shows the highest points given by the assessors.

- *Statistical Analysis*

The data was analysed by random method. Experiments included massecuit III in different levels. For each sample, 3 repetitions were carried out. Duncan

multiple range test was used in level 0.05 to compare the data. Data analysis was carried out by version 21 of SPSS software.

Friedman test was used to analyse the sensual tests.

Results and Discussion

Table 1 shows the chemical analysis of massecuit III. The results indicated that the quality of this product is within the standard. Table 2 presents the qualitative and quantitative concentrations of polyphenolic compounds that were identified by HPLC in massecuit III. The impurities such as coumaric acid or ferulic acid and other polyphenolic compounds have proved to exhibit and possess strong antioxidant activities. The presence of phenolic compounds in mediatory products of sugar factory could be related to the minimum processing and the presence of more impurities of these products as compared to the white sugar (Nakaya *et al*., 2009).

The antioxidant activities and the findings are presented in Table 3. The results are based on DPPH radical scavenging assay for massecuit III and the activity was compared with that of ascorbic acid.

DPPH radical scavenging assay on massecuit III showed that this sugar has antioxidant activity (EC_{50}=78.53). Phenolic compounds in massecuit III explain the activity against DPPH radical scavenging assay (Payet *et al*., 2005). Food components with EC_{50}>30 are weak antioxidants (Qusti *et al*., 2010) and it should be considered that since sugar is normally used in food products at different concentrations as a sweetener and brown sugar that is a mediatory product that has economical aspects has radical scavenging activity as compared with the white sugar (Payet *et al*., 2005).

Table 1. Chemical analysis of massecuit III

Humidity (%)	Lead (ppm)	Arsenic (ppm)	Solution Color (IU)	pH	Sulfated Ash (%)	Invert Sugar (%)	Polarization (oz)
0.5±0.01	<0.003	0.008	5970±10	7.4±0.1	6.1±0.02	0.75±0.01	83.8±0.06

Table 2. Identification of polyphenolic compound of massecuit III

Phenolic compounds	RT(min)	Amount (mg/kg)
p-hydroxybenzoic acid	12.8000	22.92
Vanilic acid	15.1567	10.26
Homovanilic acid	16.9833	32.33
Vanilin	20.8330	2.98
Coniferyl alcohol	21.2800	2.78
p-coumaric acid	22.1500	20.05
Acetosyringone	25.0660	18.28
Ferulic acid	27.1670	13.37
benzoic acid	28.8160	2.99
The total polyphenol content		**126.27**

Table 3. DPPH radical scavenging assay of massecuit III and acid ascorbic

Sample	DPPH (EC50 – mg/ml)
massecuit III	78.53±0.19
Ascorbic acid	3.51±0.13

The analysis of variance and the comparisons of the acidity of control and chocolate milk samples regarding different percentages of massecuit III are shown in Figure 1.

According to these findings, samples having 75% and 100% massecuit III, did not present significant differences in respect of acidity, but they showed a significant difference as compared to the control sample.

The analysis of variance in respect of the pH of the samples and the comparison with the control including different concentrations of massecuit III are shown in, Figure 2.

The significant difference between the treatments was considered as $p< 0.01$.

The analysis of variance and the comparison of the average of total count of samples and chocolate milk consisting of different percentages of massecuit III are presented in, Figure 3.

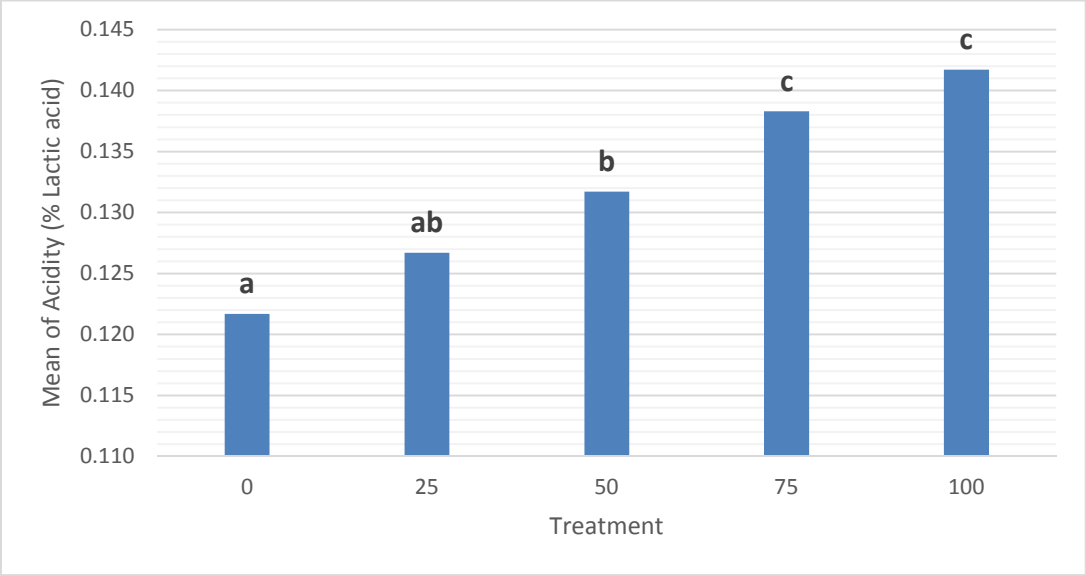

Fig. 1. The comparison of average acidity for the investigated samples

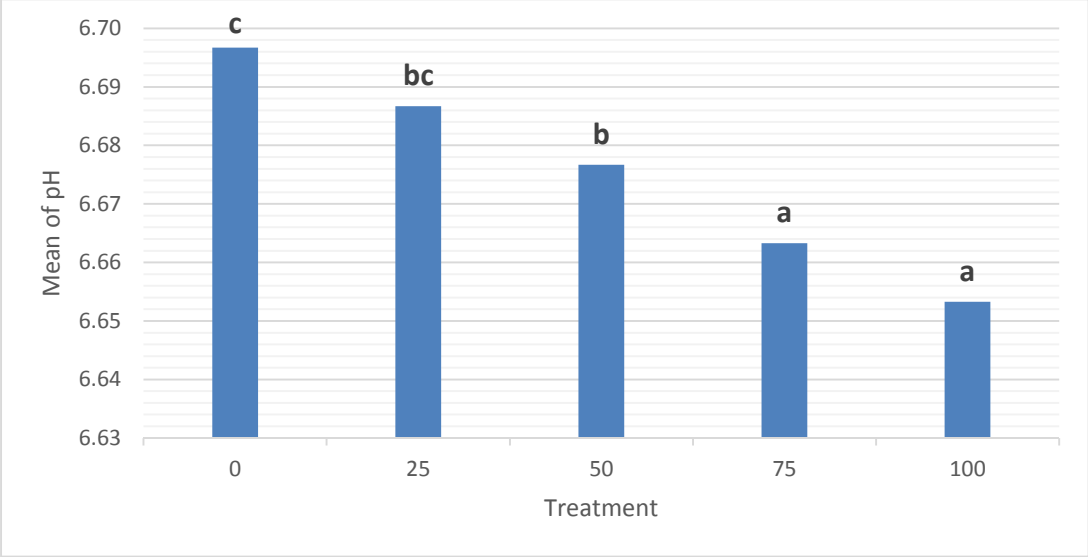

Fig. 2. The comparison of average pH for the investigated samples

The results indicated that significant differences regarding total count were not observed between the samples. Massecuit III replaced sugar at different concentrations in chocolate milk and evaluation of the acidity, pH and total bacterial count showed that all these characteristics were in the normal range in all the samples examined and were within the defined national standard of Iran.

Analysis of variance and the comparison of 'EC_{50}' values of the samples including different percentages of Massecuit III are shown in Figure 4.

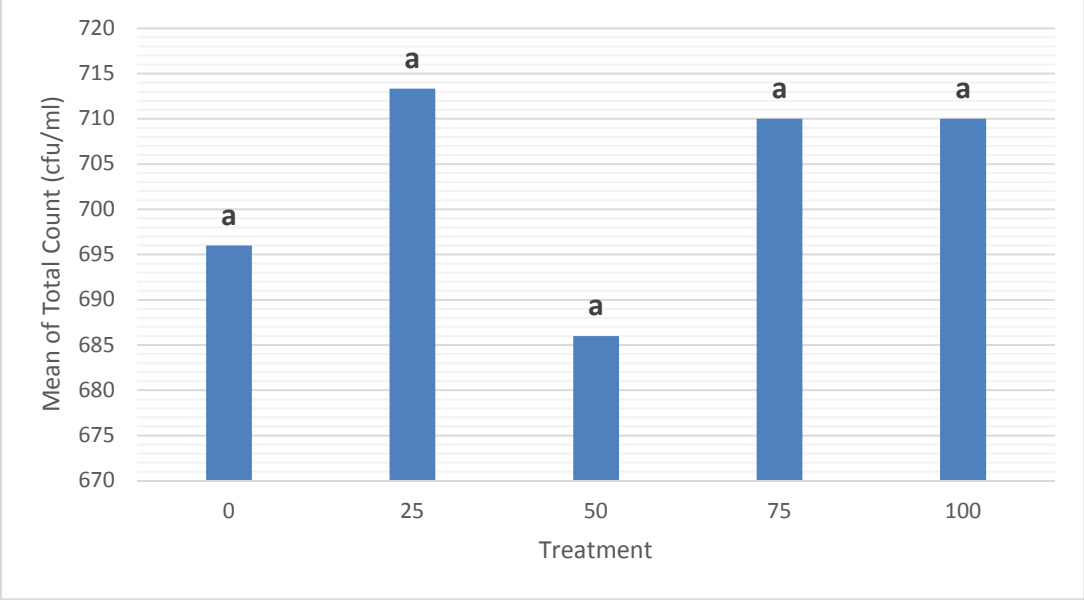

Fig. 3. The comparison of total bacterial count for the investigated samples

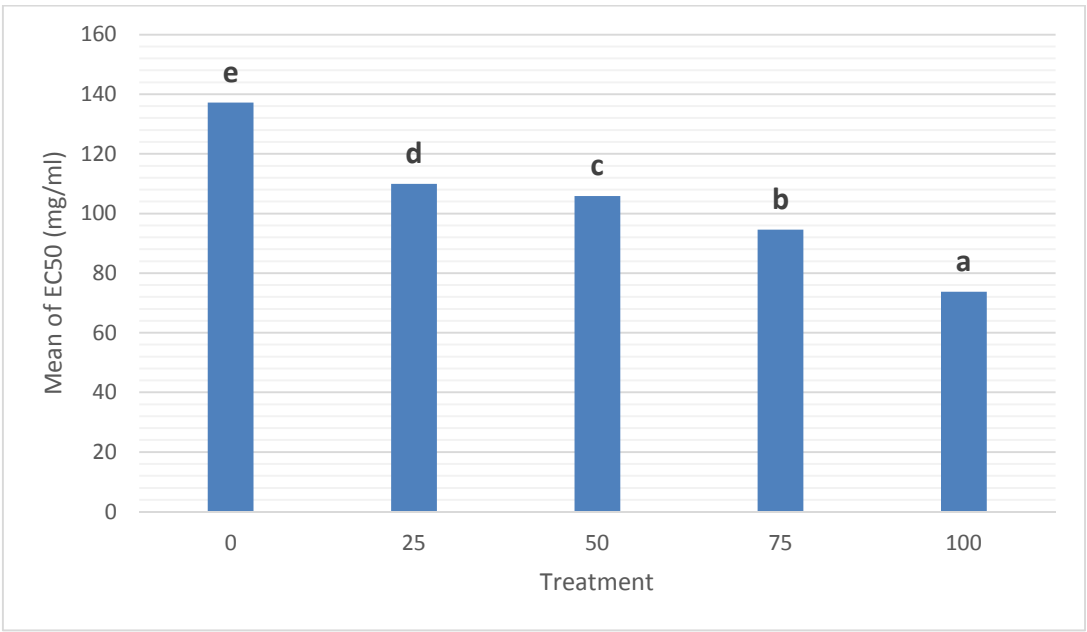

Fig. 4. The comparison of chocolate milk average EC_{50} values for the investigated samples

Regarding EC_{50} values, all the samples showed significant differences as compared with each other. The antioxidant activity of the control and the samples were evaluated by DPPH free radical scavenging assay. In order to evaluate the antioxidant activity of chocolate milk, EC_{50} factor was used. According to the definition of EC_{50} that is mg per ml is the concentration of methanol essence in which 50% of DPPH free radicals in action environment are limited. Therefore, the higher the antioxidant activity, the less the EC_{50} would be. According to the result of the data obtained, it was found that by increasing massecuit III concentration, the antioxidant activity is increased in the samples that means a decrease in EC_{50}. EC_{50} is found by linear regression in a diagram in which DPPH free radical percentage is drawn against tested concentrations (Sakak *et al*., 2011).

The significant difference between the treatments was considered as $p<0.01$. The reason for the antioxidant activity in the control group might be due to the vanilla essence and cocoa powder that might contain flavonoids such as procianidin (Sharaf o din, 2011).

Lu *et al*. (2010) in a similar research, using the same method, studied the quality of the sponge cake made of green tea. It was concluded that the DPPH free radical activity was due to the green tea.

The antioxidant activity and DPPH free radical scavenging was studied in different mineral water samples that were enriched with fruit juices and tea and herbal essence in a wide range of samples. Depending on the phenolic compounds present the antioxidant activities were observed (Lozzio *et al*., 2012).

- *Sensory evaluation of the chocolate milk*

The data presented in Table 4 and Table 5 demonstrated the findings of Friedman test and the rankings after the production. The control and the samples of chocolate milk with 25, 50, 75, 100% replacement of massecuit III have significant differences between the treatments regarding the texture ($p<0.01$) and acceptability ($p<0.05$). Therefore, ranking table is valid concerning the texture and acceptability. According to Table 5, the samples containing 25% and 50% of sugar had the highest evaluated values concerned with the texture and acceptability as compared to the control.

Table 4. Friedman test data output

Adj	Asymp. Sig.	Df	Chi-Square	Number
Color	0.159	4	6.597	7
Aroma	0.558	4	3.00	7
Taste and flavor	0.097	4	7.845	7
Texture	0.013	4	12.654	7
Sweetness	0.657	4	2.429	7
Overall acceptability	0.008	4	792.13	7

Table 5. Ranking table of texture and acceptability

Sample	Overall acceptability	texture
Control	2.43	2.79
%25	3.14	3.29
%50	4.50	3.29
%75	2.79	43.2
%100	2.14	2.07

Conclusion

Considering the low level of dairy consumption in Iran, the use of chocolate milk and similar formulated products might improve and to some extent replace the level of dairy consumption. Therefore, improvement in the quality of chocolate milk as a part of dairy chain products is necessary and essential. Sugar is considered as one of the main ingredients in chocolate milk. Massecuit III as an alternative of sugar might improve the quality of chocolate milk. The replacement of white sugar with massecuit III in the formulation of chocolate milk increases the antioxidant activity and also helps to improve the nutritional values of the product. Due to the omission of production process in massecuit III as compared to the white sugar, the total price of this product would be much lower. Therefore, by replacing white sugar with massecuit III, one might be able to produce a product with higher nutritional value and lower price and this might highly be recommended for a country such as Iran.

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