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A survey of the physicochemical properties of unsweetened chocolate enriched with whey protein, milk isolate, isomalt and grape syrup

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ABSTRACT:

One of the major problems of chocolate consumption is its high sugar. Various efforts have been made to substitute sucrose of chocolate and most of them have not been accepted due to unsuitable texture and digestive effects. This study provided chocolate by alternatives of grape syrup, isomalt whey and milk powder with 21 other formulations. Physicochemical properties like ash, sugar percent, sucrose percent, reducing sugar after hydrolysis, protein percent, sensory, rheological and microbial properties including mold and yeast contamination, total microbial and coliform counts were evaluated. The number of treatments in this study was 21. Data were analyzed using multi-range Duncan test to determine the difference between the means at the confidence interval level of 0.05. The data were analyzed using SPSS software and Excel.

The results showed that there was a significant difference between all treatments in each of the properties ($p<0.01$). Generally, in all formulations of chocolate, sucrose percent was significantly different from that of the control group ($p<0.01$). The lowest sucrose was dedicated to treatment T17 with 6% of grape syrup, 6% isomalt, 15% milk protein isolate and 15% whey protein and T21 with 6% of grape syrup, 6% isomalt, 5% milk isolate protein and 5% whey protein. The increase of whey protein and milk powder in 10 and 15% respectively, increased the ash of samples. In samples with high percentage of grape syrup and isomalt, there were significant changes in ash. The increase of each of the compounds had significant impact on the total ash of chocolate. Also, the control group had the lowest total ash. Based on the results of study regarding pH and acidity measurement, the highest pH and the lowest acidity were dedicated to the control group and adding grape syrup to chocolate, reduced pH significantly and increased acidity. The comparison of the mean at the 99% level showed that pH reduction and acidity increased in all alternatives were significant compared to the control group. Totally, the substitution percent of 2% grape and isomalt syrup, 5% of desalinated whey powder and milk power were good in optimal formulation and T1 treatment was the considered as best treatment.

Keywords:
Milk powder, Whey protein, unsweetened chocolate, Grape syrup, Isomalt

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INTRODUCTION

This chocolate is suitable for diabetic and obese people. Diabetes and obesity are common diseases and most adolescents and adults suffer from them. This problem is alleviated by unsweetened and low-calorie products to prevent blood sugar increase (Ahmadnia and Sahari, 2008). Isomalt powder or granola is a sweetener derived of sugar and its sweetening value is half of the sucrose sweetening but is similar to sugar in terms of flavor and taste. Despite artificial sweeteners, it is used as a sweetener and increasing texture of food (Chegeni, 2006). Grape syrup is a sweet matter made of grape juice. At first, grape juice was extracted and, then alkaline soil is added to it and this causes that grape pomace getting deposited with the soil, then grape juice is poured into the pots and boiled to get red-gold liquid (Eslamian, 2011). Whey powder is the product of drying water isolated from milk after the cheese production or Permeate (the powder of whey produced from UF process) to achieve moisture (3-4%). One of the most important properties of this product is solubility, density and microbial properties and lactose in crystal form keeps the quality and avoids moisture absorption (Eslamian, 2011). The protein of milk isolate is achieved by doing extra processes on concentrate protein with 85% protein. The major part of fat and carbohydrate are eliminated but casein and whey protein is intact (Eslamian, 2011).

Ahmet *et al.* (2006) evaluated the impact of some sweeteners including maltitol, isomalt, and xylitol on rheological properties. Gee *et al.* (1991) evaluated the effect of sucrose, fructose and isomalt on chocolate. In which isomalt had the highest effect on blood sugar reduction.

Farzanmehr *et al.* (2008) evaluated the impact of sugar alternatives on some physicochemical properties of milk chocolate. They found that the treatments with high sugar alternative compound showed high moisture and viscosity and yield stress and stiffness in the middle ratios of these compounds compared to the other treatments. Imamjome *et al.* (2010) evaluated the effect of sucrose and glucose alternatives with two diet sweeteners on texture and microstructural properties of Gaz. The results of texture evaluation showed that the highest similarity was found in the treatment with 25% sorbitol and 65% isomalt compared to the control treatment. The increase of sorbitol increased the texture softness. In the evaluation of texture microstructure, it was shown that the ordinary Gaz had many bubbles and this reduced its density compared to the unsweetened treatment. In alternatives of unsweetened sugar, this bubble was not observed and this increased its density compared to unsweetened treatment. Unsweetened substitutes had no bubble and this increased the product density compared to control treatment. The present study aimed to evaluate the physicochemical properties of unsweetened chocolate enriched with whey protein, milk isolate protein, isomalt and grape syrup.

Methodologies

pH measurement

It was measured by pH meter based on national standard of Iran NO. 2852 (National standard of Iran, 2008).

Acidity measurement

Acidity was measured by neutralizing with ethyl alcohol, phenolphthalein and standardized potassium hydroxide based on the national standard of Iran, NO. 608 (National standard of Iran, 2010).

Protein measurement

By AACC2000 standard NO. 10-46, protein is measured.

Sugar measurement

It is performed by national standard NO. 759.

Ash measurement

It is performed by AACC 2000 standard NO. 10-80.

Coding of the treatments are shown in Table 1.

Table 1. Coding of study treatments

Treatment code	Isomalt %	Milk isolate protein %	Whey protein %	Grape syrup %
T1	2	5	5	2
T2	4	10	10	2
T3	6	15	15	2
T4	2	10	5	2
T5	2	15	5	2
T6	4	5	5	2
T7	6	5	5	2
T8	2	5	5	4
T9	4	10	10	4
T10	6	15	15	4
T11	2	10	5	4
T12	4	5	5	4
T13	2	5	10	4
T14	6	5	5	4
T15	2	5	5	6
T16	4	10	10	6
T17	6	15	15	6
T18	2	10	5	6
T19	4	5	5	6
T20	2	5	10	6
T21	6	5	5	6
T	-------	--------	-------	------

The formulation of control sample doesn't have alkaline cocoa powder (10-12% fat), odorless powder of Kepong Malaysia, Vanilla panda-China, sugar (40.4%), and total dried milk 18%. In the above formulation and total isomalt and grape syrup were deducted from sugar percentage.

RESULTS AND DISCUSSION

As shown in Table 2, pH range of chocolate samples ranged between 4.6-5.5. The results showed that the highest pH was dedicated to control. Adding grape syrup to chocolate reduced pH significantly and it was consistent with the acidity results. Adding whey powder, milk power changed chocolate samples but in the samples with higher concentrations of grape syrup 6%, pH changes were higher. In samples 2% and higher value of milk protein and whey, there were no significant changes. Adding isomalt increased pH reduction in chocolate samples. There was no significant difference between T8 and T11 treatments with control group. The study of Kafshgari *et al.* (2014) evaluated sugar substitution with grape syrup in vanilla ice cream. The results were consistent with the present study. Isomalt in high value increased pH in chocolate samples due to acid hydrolysis and the results are consistent with the findings of Bitaraf *et al.* (2013).

The results of acidity changes were similar to that of pH. The samples with grape syrup 6% had the highest acidity. The samples with formulation of isomalt above 6% showed significant increase in chocolate.

Table 2. The results of analysis variance in pH in the chocolate treatments (p<0.05)

Value	Treatment	Value	Treatment
0.01±7.2 ^{b}	T11	0.01 7.05±^{ab}	T1
0.01±6.65^{c}	T12	0.01 7.34±^{a}	T2
0.01±6.65^{c}	T13	0.02 8.56±^{c}	T3
0.01 7.09±^{b}	T14	0.01 7.35±^{ab}	T4
0.03 7.08±^{b}	T15	0.01 7.35±^{ab}	T5
0.01 8.02±^{d}	T16	0.03±6.5^{c}	T6
0.02±8.72^{f}	T17	0.01±6.07^{c}	T7
0.02±5.25^{g}	T18	0.02 7.09±^{b}	T8
0.01±5.2^{d}	T19	0.05±5.8^{d}	T9
0.01±5.6^{d}	T20	0.05±8.8^{f}	T10
0.02 7.39±^{b}	T	0.01±5.72^{de}	T21

Table 3. The variance analysis results of acidity in the chocolate treatments (p<0.05)

Value	Treatment	Value	Treatment
0.01±6.2 ^{b}	T11	0.01 6.12±^{ab}	T1
0.01±5.65^{c}	T12	0.01 6.34±^{a}	T2
0.01±5.65^{c}	T13	0.02 6.56±^{c}	T3
0.01 6.29±^{b}	T14	0.01 4.35±^{ab}	T4
0.03 7.28±^{b}	T15	0.01 6.35±^{ab}	T5
0.01 7.02±^{d}	T16	0.03±5.5^{c}	T6
0.02±7.72^{f}	T17	0.01±5.07^{c}	T7
0.02±4.25^{g}	T18	0.02 6.09±^{b}	T8
0.02 7.87±^{gh}	T19	0.05±5.8^{d}	T9
0.02 7.81±^{gh}	T20	0.05±7.8^{f}	T10
0.01±55.5^{a}	T	0.03± 8.86^{h}	T21

Table 4. The results of variance analysis of moisture at variable chocolate treatments (p<0.05)

Value	Treatment	Value	Treatment
0.23 7.2±^{e}	T11	0.02 4.21±^{a}	T1
0.23 7.5±^{e}	T12	0.02 3.21±^{e}	T2
0.02 4.41±^{a}	T13	0.02 3.81±^{e}	T3
0.02±5.55^{f}	T14	0.01 4.01±^{c}	T4
0.02 4.11±^{b}	T5	0.02 4.11±^{b}	T5
0.01 7.29±^{e}	T16	0.02 5.25±^{a}	T6
0.02±8.11^{d}	T17	0.02 3.81±^{e}	T7
0.23 7.5±^{e}	T18	0.02 4.41±^{a}	T8
0.09±8.2^{h}	T19	0.02 4.59±^{a}	T9
0.01 9.71±^{g}	T20	0.01 4.01±^{c}	T10
0.02 4.41±^{a}	T	0.03±4.14^{a}	T21

Table 5. The variance analysis of protein in different chocolate treatments (P<0.05)

Value	Treatment	Value	Treatment
0.03±5.25[b]	T11	0.01±5.46[ab]	T1
0.01±5.46[ab]	T12	0.02±5.87[c]	T2
0.03±5.25[b]	T13	0.01±8.85[e]	T3
0.01±5.46[ab]	T14	0.03±5.25[b]	T4
0.01 7.29±[f]	T15	0.02±5.87[c]	T5
0.02±5.87[c]	T16	0.01±5.46[ab]	T6
0.01 8.85±[d]	T17	0.01±5.46[ab]	T7
0.03±5.25[b]	T18	0.01±5.46[ab]	T8
0.01±5.46[ab]	T19	0.02±5.87[c]	T9
0.03±5.25[b]	T20	0.01 8.85±[d]	T10
0.01±5.18[a]	T	0.01±5.46[ab]	T21

Adding whey isolate and milk powder didn't create significant changes in chocolate samples. Only at high percentage of protein isolate, acidity was reduced significantly. The results were consistent with the findings of Kafshgari *et al.* (2014).

The results showed that whey protein reduced moisture in chocolate samples. In isomalt samples, 2% substitute increased moisture but excessive use reduced significantly compared to control group with sucrose. In grape syrup samples, the moisture was increased compared to that of control group. The sample with grape syrup at 6% had higher moisture compared to control group. The highest moisture was reported in T16, T17 samples. In the sample T20, by simultaneous increase of grape syrup and whey protein powder, there was no significant difference compared to the control group. The increase of isomalt percentage at values 4, 6% like treatments T6 and T7 reduced moisture of chocolate samples considerably compared to the control

Table 6. The results of variance analysis of sugar in different chocolate treatments (p<0.05)

Value	Treatment	Value	Treatment
0.01 0.59±[d]	T11	0.01 0.49±[a]	T1
0.02±0.53[b]	T12	0.02±0.53[b]	T2
0.01 0.59±[d]	T13	0.01 0.65±[c]	T3
0.01 0.78±[f]	T14	0.01 0.49±[a]	T4
0.02±0.63[g]	T15	0.01 0.49±[a]	T5
0.04±0.73[h]	T16	0.02±0.53[b]	T6
0.01 0.81±[f]	T17	0.01 0.65±[c]	T7
0.02±0.63[g]	T18	0.01 0.59±[d]	T8
0.04±0.73[h]	T19	0.02±0.64[e]	T9
0.02±0.63[g]	T20	0.01 0.78±[f]	T10
0.01 0.81±[f]	T	0.01 0.81±[f]	T21

Table 7. The results of variance analysis of sucrose of chocolate treatments (p<0.05)

Value	Treatment	Value	Treatment
0.01 0.21±[d]	T11	0.01 0.23±[a]	T1
0.02±0.26[e]	T12	0.02±0.18[b]	T2
0.01 0.21±[d]	T13	0.01 0.13±[c]	T3
0.01 0.11±[f]	T14	0.01 0.23±[a]	T4
0.02±0.19[g]	T15	0.01 0.23±[a]	T5
0.04±0.14[h]	T16	0.02±0.22[b]	T6
0.01 0.9±[f]	T17	0.01 0.23±[c]	T7
0.02±0.19[g]	T18	0.01 0.21±[d]	T8
0.04±0.14[h]	T19	0.02±0.16[e]	T9
0.02±0.19[g]	T20	0.01 0.11±[f]	T10
0.01 0.30±[a]	T	0.01 0.9±[f]	T21

group. In treatments T2 and T3 by the increase of milk powder and whey protein and isomalt to 4%, moisture was reduced. This is due to the presence of alcohol sugar of isomalt in chocolate formulation. The alcohol sugar with hydroxyl group can have hydroxyl groups and water in their formulation. The results of this study were consistent with the findings of Jeli *et al.* (2013) in using isomaltose and sucralose instead of sucrose. The increase of isomalt percentage in the formation of above 4% reduced moisture compared to that of control group. Sucrose sugar has high moisture retention compared to isomalt alcohol sugar. Alcohol sugar has compressed structure but sucrose molecular structure is discrete (Jeli *et al.,* 2013).

As shown in Table 5, the increase of grape syrup and isomalt had no significant impact on protein of chocolate samples. By increase of whey protein percent, the protein percent of chocolate samples increased significantly. The increase of whey protein percent and milk powder showed significant difference in treatments. In treatments with milk powder and whey protein with equal percentage, chocolate protein didn't show significant difference. Silveira *et al.* (2015) evaluated and found that whey powder increased protein percent in dairy beverage.

As shown in Table 6, in unsweetened chocolates, by increase of isomalt and grape syrup, total sugar percentage was increased. In 2% grape syrup and 2% isomalt, the changes in total sugar were not significant.

Table 8. The results of variance analysis of reducing sugar after hydrolysis of chocolate treatments ($p<0.05$)

Value	Treatment	Value	Treatment
0.01 0.21±[d]	T11	0.01 0.23±[a]	T1
0.02±0.26[e]	T12	0.02±0.18[b]	T2
0.01 0.21±[d]	T13	0.01 0.13±[c]	T3
0.01 0.11±[f]	T14	0.01 0.23±[a]	T4
0.02±0.19[g]	T15	0.01 0.23±[a]	T5
0.04±0.14[h]	T16	0.02±0.22[b]	T6
0.01 0.9±[f]	T17	0.01 0.23±[c]	T7
0.02±0.19[g]	T18	0.01 0.21±[d]	T8
0.04±0.14[h]	T19	0.02±0.16[e]	T9
0.02±0.19[g]	T20	0.01 0.11±[f]	T10
0.01 0.9±[f]	T21	0.01 0.16±[a]	T

The increase of whey protein had no significant impact on any of the samples. The increase of percentage of isomalt and grape syrup increased total sugar but it reduced sucrose. The results of the study were consistent with the findings of Farzanmehr *et al.* (2008). They evaluated the effect of sugar substitutes on some physicochemical, rheological and sensory properties in milk chocolate. They found that inulin, polydextrose and sucralose increased total sugar percent to 10%.

As shown in Table 7, in all formulations of chocolate, sucrose percent had significant difference with that of control group. The lowest sucrose in formulation was dedicated to T21 and T17 treatments. T1, T5, T4 and T7 had no significant difference with each other. The results of the study of Kieran Keogh *et al.* (2003) were found to be consistent with the present study. They evaluated the effects of using dried milk powder by ultrasound method on some properties of milk chocolate and found that dried milk powder had no effect on milk chocolate properties.

The changes of reducing sugar after hydrolysis of chocolate samples were like the sugar percentage and by the increase of whey protein powder and milk powder they too increased significantly. The lowest value of reducing sugar was dedicated to the control group. The samples with 15% whey protein and milk powder had the highest reducing sugar after hydrolysis. Sugar measurement after hydrolysis showed full acidy hydrolysis of milk protein and whey powder and this is shown in the study of Shuride *et al.* (2012).

Table 9 shows the changes of ash of chocolate samples with different formulations. As shown in the table, the increase of protein and milk powder with above 10 and 15% significantly increased ash content of the samples. In samples with high percentage of grape and isomalt extract, there were significant changes in ash. The increase of each of compounds had significant effect on total ash of chocolate samples. Also, control sample had the lowest total ash. The findings of Tavakolipour and Ashtari, (2013) regarding the grape syrup properties showed that pectic compounds of grape syrup in products formulation led into the increase of ash and the results are consistent with the findings of the study.

CONCLUSION

Generally, in formulations of chocolate, sucrose percentage was significantly different from that of control group. The lowest sucrose in formulation was dedicated to T21 and T17 treatments. Treatments T1, T5, T4 and T7 had no significant difference with each other. The increase of percentage of whey protein and milk powder with ratios above 10 and 15% significantly increased ash of samples. In the samples with high percent of grape syrup and isomalt, there were significant changes in ash of samples. The increase of each of compounds showed significant effect on total ash of chocolate samples. Also, the control sample had the lowest total ash. Based on the results of pH and acidity measurement, the highest pH and lowest acidity were dedicated to control sample and adding grape syrup to chocolate reduced pH significantly and increased acidity. The mean comparison at the level 99% showed that pH reduction and acidity increased in all substitutions and were significantly compared to the control group but these changes were not significant between the two levels 4 and 6%.

Finally, by considering all physicochemical, sensory and rheological factors, T1 treatment was recognized as the optimal treatment.

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