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Full Length Article

Stability of lemon beer containing stevia and isomalt sweeteners during storage period

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ABSTRACT
Sucrose replacement with stevia and isomalt provides the possibility to create low calorie products. The objective of the present study was to produce reduced-sucrose containing stevia and isomalt with suitable quality. The study was conducted by changing the formulation of lemon beer so that, sucrose was replaced with the ratios of 25% and 50% stevia and 25% and 50% isomalt. Then, their chemical traits including acidity, color, total sugar, turbidity, total solids, pH, brix, density and sensory attributes (aroma, taste, sweetness, fixed taste, being natural, consistency, color, turbidity and appearance, bitter after taste, overall acceptability) were analyzed in the beers stored in a n 8 ^{0}C fridge for at first after 15 and then 30 days after production time, and the results were compared to the control sample containing sucrose produced and stored in the same conditions. Results were analyzed by statistical design of completely randomized blocks, and Duncan's test was used for the means comparison. The results indicated that, by using stevia and isomalt in beer, significant changes ($p<0.05$) occur in acidity, total sugar, turbidity, pH, and brix. By replacing sucrose, a significant change was observed in aroma in the beers ($p<0.05$). Storage caused significant changes in acidity, total sugar, turbidity, pH and brix ($p<0.05$). The color of lemon beers remained fixed at all stages and was equal to 7.4 EBC. Thus, the results suggest that, replacing the sucrose in lemon beer with 25% stevia, 50% isomalt and 25% sucrose would has no effect on qualitative traits and taste of the beer and therefore,and reduced the amount of calorie of the beers.
Keywords: *Stevia, Isomalt, Sucrose, Beer, Natural sweeteners, Low calorie.*

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INTRODUCTION

By changing the culture of life and loss of physical movement human and occurring some problems such asobesity, high blood pressure, etc. and on the other hand, some diseases such as diabetes, the issue of using sucrose and its derivatives such as glucose in producing feed products for the mentioned people is becoming clearer and the necessity of resolving this issue has become more serious [1]. Awareness of people about the effectiveness of suitable feeding on their health has caused that, the producers of food products produce low fat, sugar and salt products and higher fiber which can prevent some diseases occurrence [2].

Nowadays, stevia sweetener which is a composition with natural origin, has been welcomed seriously in many countries. Despite artificial sweeteners, this sweetener is not toxic and carcinogenic. Stevia is a 100% natural sweetener without calorie and is non-fermentable [3].

Stevia leaf contains a complex blend of sweet diterpene glycosides. The sweetness of this material has been estimated by 300 times more than sugar [9]. Also, stevia has a stable taste and sweetness which can be kept at all production stages. JECFA mentioned clearly that, this sweetener is not toxic and carcinogenic. This sweetener has been confirmed by CAC, CODEX, FAO, and the World Health Organizationto use among various forms of the product. Allowed amount of daily consumption is 4 mg per kg of the body weight .

Isomalt is a type of polyols that is used in sugar-free foods formulation. The advantage of using polyols is their low calorie and natural taste; moreover, these polyols can be used by diabetic people without

increase of blood sugar and lactic acid [1]. Isomalt is achieved from sucrose within two stages: first, by an enzymatic reaction, sucrose is converted to isomal to lose which is a regenerative disaccharide. The achieved isomaltolose is hydrogenated at the presence of Nickel and is converted to isomalt. Isomalt is white, odorless, crystalline form, highly soluble, resistant to temperature and resistant to browning reactions against sucrose. This sweetener is metabolized in the body very low, and is non-absorbable, and produces only 2 kcal energy per gram [1]. Sweetness strength of isomalt is 0.45-0.6 compared to sucrose (=1) [10]. Isomalt has a pure sweet taste similar to sucrose and amplifies the severity of taste inthe foods (McNutt, 1998). Safety and health of isomalt has been confirmed in 1986. It has been also recognized as a safe additive in 70 countries around the world including Iran and is used in various industries.

Beer is a carbonated soft drink which is from barley malt, water, hops and permitted additives without the alcoholic fermentation or without added sugar [8]. Group B Vitamins, minerals particularly calcium and phosphorus, amino acids and protein are considered as the most important nutrient compounds of beer[9]. Beer is a very popular product many kinds of which contain high sucrose and calorie and can be noted as a perfect choice to transmit stevia and isomalt as the alternatives of sucrose. In a study, stevioside was used in peach juice instead of sucrose. The results of this research showed that, 34 g/l of sucrose was replaced by 160 mg/l of stevioside in the juice with 25% calorie reduction and without having any impact on organoleptic characteristics of the product. In another research, the effect of isomalt was investigated on the hydrolyzed hydrogenated starch in the sugar free caramel popcorn. The results of this study state that, the formulation with 100% isomaltis a perfect formulation through physical, chemical, and organoleptic analysis. Cardoso andBolini (2008) explained descriptive characteristics of the peach nectar sweetened by sucrose and various other sweeteners. Also, Homayounirad et al. (2012) evaluated the effect of inulin and stevia replacement on physical characteristics of milk chocolate. In this research, sucrose was replaced by stevia and isomalt at five different levels in the beer formulation, and the effect of this replacement was investigated on acidity, color, total sugar, turbidity, total solids, pH, Brix, specific gravity and organoleptic characteristics with aim to select appropriate lemon beer with reduced sucrose.

MATERIALS AND METHODS

Preparation of samples

Malt extract which contains hops, additives including food acids(lactic andcitric), vitamin C and sodiummetabisulphite(an antioxidant), was prepared from Behnoush Company before adding sugar and after filtration stage. Otherraw materialsused in the preparation of beer included Stevia (Takfa Co.), isomalt(Helmi Co.), sugar (Iran sugar), caramel food color(ESCO Company), lemonessence(Dehloran Co.). Stevia had a pH by 4.5-7, and isomalt contained 98% GPM+GPS content, 5% water (crystal), 0.5% D-mannitol, 0.5% D-Sorbitol and 0.05% ash according to the given characteristics by the producer company. Lemon beer formulation has been given in Table 1. The malt extract filtered by brix 7 was diluted by RO water to reach to brix 3.7. Then, caramel food color was added to by 0.3 g/l to achieve the considered color and finally, lemon essence was added by 0.6 g/l. Various percentages of stevia, isomalt and sugar were added to the base solution according to Table 1 and three replications were considered for each treatment.

Table 1. Formulations of control lemon beer and the beers with reduced calorie containing stevia and isomalt (stevia (sweetness percentage equal to sucrose), isomalt (sweetness percentage equal to sucrose))

	L_5	L_4	L_3	L_2	L_1
stevia (sweetness percentage equal to sucrose),	50	25	50	25	0
isomalt (sweetness percentage equal to sucrose))	50	50	25	25	0
Sugar	0	25	25	50	100

Then, the samples were thrown in PET bottles, and in order to add CO_2, dry ice was used at a rate of 1.6 gr for each 330 PET bottle. Therefore, 1.6 gr of dry ice was added to each sample and the bottles were immediately capped to prevent the removalof CO_2. Finally, the samples were pasteurized at temperature of 68 ^{0}C for 42 minutes and kept at 8 ^{0}C.

Measurement of beer samples characteristics

The beer samples pH was calculated through the methodadopted byIran National StandardNo.2280 by an electronic pH meter [4]. Brix was calculated using the methodadopted byIran National StandardNo.2280

by an ESRdevice[4]. The beer samples density was measured the method adopted by Iran National StandardNo.2280 by a hydrometer [5]. Total sugar of the samples was measured using the method adopted by Iran National StandardNo.2280 [5]. Turbidity of the samples was measured using the method adopted by Iran National StandardNo.2280 by a turbidity meter [6]. Total solids was calculated using the method adopted byIran National StandardNo.2280[6]. The measurement experiments of color, total sugar, turbidity, totalsolids, pH, Brix, specific gravity were conducted in three replications for each sample. For organoleptic evaluation of reduced calorie lemon beer using stevia and isomalt sweeteners, organoleptic evaluation method of five-point Hedonic was used by nine trainedvaluators of Behnoush Company of Iran. The coded samples were given to the valuators. In this evaluation, 10 characteristics were considered including drink tasting, smelling, sweetness, sourness, taste consistency, color, thickness, turbidity and appearance of the product, bitter taste and overall acceptance for which excellent quality to weak quality were used for the amount of liking or disliking of the samples; so that, scores of 1 to 5 were given to weak quality and excellent quality respectively.

Statistical analysis

All the experiments were conducted in three replications according to Table 1. To analyze the data obtained from laboratory methods and organoleptic tests, GLM method of SAS 9.1 software and variance analysis were used. The used layout was completely randomized blocks in which the effect of time was considered as block. After variance analysis, Duncan method was used for mean comparison of the factors. All graphs were drawn using Excel software.

RESULTS AND DISCUSSION

The considered characteristics were investigated at various replacement ratio and were compared to the control samples containing sucrose. Also, change in physicochemical traits of the drinks were evaluated during the storage period, and obtained results are as below.

As it is seen in Table 2, pH of the samples containing stevia and isomalt and control samples was significantly reduced. These effects can be due to yeasts growth and activity during storage period as well as imperfect pasteurization and capping. Swelling of the bottles containing beer after 30 days maintenance in an 8 ^{0}C fridge is the evidence of yeasts growth during the storage period. Mirzaei*et al*[11] investigated the stability of carbonated soft drinks containing sucrose and fructose-rich corn syrup during the storage period. They found that, the amount of pH has been increased during the storage period which is consistent with the present study. Also the researches about fermented beverages showed that, pH has been increased from 0.1 to 0.4 during the storage period as the result of yeasts activities (Adegoke*et al*., 1995).

The results of the color of lemon beer treatments showed that the color amount of all treatments is stable until one month maintenance and there was no difference among the control samples and the samples containing stevia and isomalt sweeteners. It is needed to be mentioned that, the amount of color was constant by 7.4 EBC in all the samples. In another research, the effect of stevioside and sucralose replacement was studied on rheological properties, color and micro-structural propertiesof mangojam. It was found that, the amount of color was not changed after production and during the storage period (Basu*et al*., 2012). Also, Mirzaei et al[11] studied the stability of carbonated soft drinks and concluded that, the color of orange and cold drinks was stable during the storage period for four months and had no significant change.

As it has been given in Table 3, total sugar of all samples containing stevia and isomalt and control samples has been increased significantly during the storage period which is due to acidic pH of the beer during the maintenance, and also, sucrose sugar which is a disaccharide, is formed by a glucose molecule and a fructose molecule, and also, stevia and isomalt sweeteners are broken and decomposed, cause to increase total sugar. In a research, the effect of drinks containing sucrose or HFCS was investigated on hunger, satiety and energy intake at the nextmeal. The researchers found that, when sucrose was hydrolyzed, its concentration was reduced from 36% of total sugar in June 30th to 10% three months later, and also, free fructose was increased from 32% to 44% which indicates that, the amount of sucrose has been reduced (Monsivais*et al*., 2007). Shachman (2005) stated that, sucrose is disaccharide composed by glucose and fructose with chemical formulation C12H22O11. Chemical bond between glucose and fructose can be broken by several ways which convert sucrose to two monosaccharides glucose and fructose. One of these methods is acidic hydrolyze in which sucrose is broken in acidic pH of drinks and under acidic conditions and then, the total sugar is increased. Acidic hydrolyze of sugar is considered as a natural phenomenon and is not a task in drink technology. Also, Rupner*et al*. (2000) produced new whey using a plant in India, and they found that, the amount of total sugar has been increases by increase of water dissolved solids.

According to Table 4, turbidity of the samples containing stevia and isomalt and control samples has been increased significantly during the storage period, it means that, the minimum turbidity was observed at the beginning and the maximum amount of turbidity was observed at 30th day which can be due to incomplete pasteurization and consequently microorganism growth, existence of hardness in the used water or existence of impurities in the used sugar, consequence of which causes to make turbidity during the product storage period.

Table 2. Acidity variations of lemon beer containing stevia and isomaltover the time

30th day	15th day	Beginning	treatments
0/40±0/011^{a}	0/34±0/005^{e}	0/33±0/010^{f}	L_1
0/40±0/005^{ab}	0/35±0/005^{e}	0/34±0/000^{ef}	L_2
0/40±0/005^{ab}	0/37±0/005^{d}	0/35±0/005^{e}	L_3
0/40±0/011^{a}	0/38±0/005^{cd}	0/35±0/011^{e}	L_4
0/40±0/005^{ab}	0/39±0/010^{bc}	0/37±0/005^{d}	L_5

- The values shown by different letters, have significant difference to each other ($p<0.05$)

Table 3. Total sugar variations of lemon beer containing stevia and isomalt over the time

- The values shown by different letters, have significant difference to each other ($p<0.05$)

30th day	15th day	Beginning	treatments
7/21±0/280^{a}	5/62±0/122^{b}	5/60±0/133^{b}	L_1
5/12±0/236^{c}	3/82±0/070^{e}	3/80±0/066^{e}	L_2
4/97±0/020^{c}	3/40±0/015^{f}	3/39±0/066^{f}	L_3
4/28±0/081^{d}	3/18±0/072^{g}	3/16±0/045^{g}	L_4
2/87±0/121^{h}	1/85±0/075^{i}	1/85±0/047^{i}	L_5

Table 4. Turbidity variations of lemon beer containing stevia and isomalt over the time

- The values shown by different letters, have significant difference to each other ($p<0.05$)

30th day	15th day	Beginning	treatments
^{a}1/28±0/036	^{a}1/17±0/050	^{a}1/06±0/020	L_1
^{a}1/64±0/050	^{a}1/54±0/045	^{a}1/31±0/020	L_2
^{a}1/35±0/061	^{a}1/27±0/025	^{a}1/39±0/441	L_3
^{a}1/63±0/090	^{a}1/48±0/032	^{a}1/42±0/025	L_4
^{a}1/83±0/030	^{a}1/74±0/051	^{a}1/65±0/040	L_5

Maqsoudi mentioned that, despite being transparent, a turbidity or deposit is created in the juices or other drinks during the storage after filling the container by drink, this phenomenon is called secondary turbidity. The cause of this phenomenon is not only insufficient transparency act, but also, water(due to having metal ions), sugar(due to the existence of saponins) and imperfect pasteurization (due to microorganisms' growth). Also, according to national standard of Iran, if there are enough mesophilicbacteriaand yeast in the product, they can be adopted to the drink environment and be reproduced. Their growth is usually along with regenerating deposit, turbidity and gas production [4]. Elhamirad and Mohammadi[2] evaluated the formulation of carbonated drinks and its physicochemical and microbiological variations during the storage period. They concluded that, temporal and environmental conditions have had significant effect on the amount of turbidity; so that, the amount of turbidity was increased in the samples maintained in transparent bottles and temperature of the environment. Loureiroand Querol (1999) evaluated the prevalence and control of spoilage yeasts in food and beverages. They found that, production of gas and turbidity is as the result of yeasts growth in the drinks during storage period.

According to Table 5, total solids of the samples containing stevia and isomalt and control samples showed no significant difference during the storage period for 30 days.

According to Table 6, pH of the samples containing stevia and isomalt and control samples has been reduced significantly. These effects can be due to the growth and activity of yeasts during the storage period as well as imperfect capping and pasteurization during which acidity is increased and pH is decreased. Baqaei et al[3] investigated the formulation of orange drink based on cantaloupeseed during

the storage period. They found that, storage period had a significant effect on the samples pH so that, pH had a descending trend. They considered the growth ofacid-producing microorganisms as the cause of this task. In another research, Baatey et al. (2002) studied spoilage modeling of *Saccharomyces cerevisiae* and candida lipolytica in cold-filled drink. They found that, spoil age yeasts can cause pH reduction during the storage period. The researchers' studies on fermented beveragesshowed that, thepH has been decreased after 48 h kept maintenance from 5.1 to 4.3. Mirzaei et al[11] also conducted a study on the stability of carbonated soft drinks and indicated that, pH of the samples containing fructose and control samples has been significantly reduced during the storage period caused by two temperature and time factors. This effect can be justified by yeasts growth so that, they cause to reduce the samples pH by consuming the sugar existing in the drinks and then acid production, cause to reduce the samples pH. But, the conducted studies about carbonated drinks with mango taste containing 5%, 7% and 10% mango pulp showed that, vitamin C has been reduced significantly, while, pH and acidity had no significant change which is in conflict with the results of the present research that is probably due to the lack of yeasts growth in the drink with mango taste.

According to Table 7, water dissolved solids in the samples containing stevia and isomalt and control samples had no significant change during the storage periodbut, they were increased insignificantly. In the low pH and acidic environment of the beer, acidic hydrolyze of the sugar existing in the beer causes to increase total sugar and consequently water dissolved solids during the storage period. Investigators evaluated the Brix degree and sorbitol and xylitolin pomegranate juice. The amount of sorbitolandxylitol of pomegranate juice was between 16 and 423 mg/l. Also, Brix degree of natural pomegranate juice containing sorbitolandxylitol has been increased from 12.2 to 17.8 (Turkmen and Eksi, 2011). In another research, the investigators evaluated the use of stevia as an alternative of sugar in the drinks. They concluded that, the content of water dissolved solids is directly related to the amount of sugar, and has been increased during the storage period (SaniahandSamsiah, 2012).

Table 5. Total solids variations of lemon beer containing stevia and isomalt over the time

30th day	15th day	Beginning	treatments
a9/13±0/057	a9/20±0/100	a9/23±0/152	L_1
a8/83±0/115	115a8/93±0/	a8/90±0/100	L_2
a7/60±0/173	a7/53±0/251	a7/50±0/264	L_3
a9/53±0/152	a9/60±0/264	a9/66±0/208	L_4
a8/70±0/100	a8/66±0/057	a8/60±0/100	L_5

- The values shown by the same letters, have no significant difference to each other (p>0.05)

Table 6.pH variations of lemon beer containing stevia and isomalt over the time

30th day	15th day	Beginning	treatments
i3/20±0/005	g3/28±0/000	ab3/31±0/000	L_1
i3/20±0/000	h3/27±0/010	de3/29±0/005	L_2
ij3/19±0/005	ef3/29±0/000	cd3/30±0/000	L_3
j3/19±0/000	ef3/29±0/000	bc3/30±0/005	L_4
ij3/19±0/005	fg3/28±0/005	a3/31±0/005	L_5

- The values shown by different letters, have significant difference to each other (p<0.05)

Table 7.Water dissolved solids variations of lemon beer containing stevia and isomalt over the time

30th day	15th day	Beginning	treatments
a0/057 ±8/23	a0/000 ±8/00	a0/000 ±8/00	L_1
a0/057 ±8/03	a0/000 ±7/70	a0/000 ±7/70	L_2
a0/100 ±7/10	a0/000 ±6/80	a0/000 ±6/80	L_3
a0/057 ±8/86	a0/000 ±8/60	a0/000 ±8/60	L_4
a0/057 ±8/03	a0/000 ±7/70	a0/000 ±7/70	L_5

- The values shown by the same letters, have no significant difference to each other (p>0.05)

Table 8.Density variations of lemon beer containing stevia and isomalt over the time

30th day	15th day	Beginning	treatments
a0/100 ±3/10	a0/152 ±3/13	a0/057 ±3/03	L_1
a0/000 ±3/00	a0/057 ±3/03	a0/000 ±3/00	L_2
a0/000 ±3/00	a0/057 ±3/03	a0/000 ±3/00	L_3
a0/115 ±3/06	a0/115 ±3/06	a0/100 ±3/10	L_4
a0/000 ±3/00	a0/100 ±3/10	a0/057 ±3/03	L_5

- The values shown by the same letters, have no significant difference to each other (p>0.05)

According to Table 8, the samples' density had no significant change during the storage period. Results obtained from evaluation of organoleptic evaluation of treatments containing lemon beer showed that, all the studied traits had no significant difference.

CONCLUSION

According to the obtained results, treatment L_4 (containing 25% stevia, 50% isomalt and 25% sugar) had the highest effect after the control treatment on the investigated organoleptic traits. So, the superior treatment is L_4. Thus, in terms of the studied traits, beer L_4 is an appropriate alternative for the beer with reduced-calorie containing stevia and isomalt sweeteners. Considering the advantage of using such kind of beer in terms of health, it is suggested to consider other sweeteners to improve taste and aroma.

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