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Full Length Article

Isotonic beer production

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ABSTRACT
In this research, the possibility of isotonic beer production has been investigated. Filtered malt extract was used and 20 treatments with various percentages of minerals calcium chloride were prepared in five concentrations including 33.16, 30, 20, 10 and 6.86 mg/100 cc, magnesium chloride in five concentrations including 5.55, 3.25, 5, 1.50 and 0.95 mg/100cc, sodium chloride with concentrations of 7.11, 70, 47.50, 25 and 17.98 mg/100cc and vitamin C with a constant amount by 15 mg. the treatments were produced considering required minerals and vitamins and investigation of their presence and absence in analysis of primary malt extract and the determined permitted limit for additives. The treatments were analyzed using statistical method RSM. Physicochemical tests and sensory evaluation of formulated drinks were investigated. The effect of calcium, magnesium and sodium was significant on osmotic pressure, turbidity acidity, pH, and density (P<0.05) and it was not significant on color and brix (P>0.05). Considering the importance of turbidity and osmotic pressure and investigating these factors in the treatments and doing Forcing test within 30 and 60 days and sensory evaluation, treatment B with formulation of calcium: 10mg/100cc, magnesium: 5mg/100cc, sodium: 25mg/100cc and malt extract: 50mg/100ccwas chosen as the best treatment.
Keywords: Isotonic beer, osmotic pressure, turbidity, statistical method RSM

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INTRODUCTION

Sports drink is a soft drink containing sugar and different electrolytes which helps to replace lost water, energy and minerals and is categorized to sports, isotonic, hypertonic and hypotonic drinks. Sports drinks increase sports performance as well as preservation of glycogen stores after exercise [7]. Sports drinks reduce physiological cardiovascular and stresses of neuromuscular systems of the athletes. Sports drinks are fortified by carbohydrate 4-8%, sodium and potassium. Sports drinks preserve blood glucose level [1]. Nowadays, considering adverse effects of sports drinks such as the existence of some compounds including caffeine, carbon dioxide and non-natural additives, natural resources are used to produce sports drinks [3]. Considering the mentioned items, sports drink production based on beer is very important. Despite the drinks which are found nowadays abundantly, beer is extracted from natural material and has countless useful properties and contains various vitamins and solute [20]. Beer is a carbonated and non-alcoholic drink which is produced from barley malt, water, hops and permitted additives without alcoholic fermentation. The beer color is golden and clear and its density is almost equal to water because, 80% of beer is water. Beer wards off kidney stones. This drink has various vitamins of group B, Pantothenic acid, biotin, folic acid and vitamin C. Beer causes to eliminate stress and many other neurological disorders particularly after exercise.
Mineral compound dissolved in water and body liquids which have positive charge (cations) and negative (anion)are called electrolyte. The ions sodium, potassium, claire, etc. of the electrolytes are the effective factors to regulate the body's water balance. There are two types of minerals: macro minerals and negligible minerals. Body needs more to macro minerals. Macro minerals include: calcium, magnesium, sodium, potassium, chloride and phosphorus. Negligible minerals include: iron, manganese, copper, iodine, zinc, cobalt and selenium [10]. In this research, the possibility of sports drink production has been investigated by adding solute and minerals including chloride, sodium, potassium, magnesium and vitamin C to the beer.

MATERIALS AND METHODS

Malt extract is the main material used in this research which is achieved after mill, dough preparation, drawing extraction, boiling and filtration. Also, other used materials in this research include: sucrose (sugar Varamin), sodium chloride (Armin), magnesium chloride (Armin) Calcium Chloride (APC Save China) Vitamin C (M.P biomedical of Netherlands).

Table 1-Characteristicsof the study treatments

Vitamin C mg/cc100	Na mg/cc100	Mg mg/cc100	Ca mg/cc100	treatments
15.00	17.89	3.25	20.00	A
15.00	25.00	5.00	10.00	B
15.00	25.00	5.00	30.00	C
15.00	25.00	1.50	30.00	D
15.00	25.00	1.50	10.00	E
15.00	47.50	3.25	20.00	F
15.00	47.50	3.25	20.00	G
15.00	47.50	0.95	20.00	H
15.00	47.50	3.25	20.00	I
15.00	47.50	3.25	6.86	J
15.00	47.50	3.25	33.16	K
15.00	47.50	3.25	20.00	L
15.00	47.50	3.25	20.00	M
15.00	47.50	5.55	20.00	N
15.00	47.50	3.25	20.00	O
15.00	70.00	5.00	30.00	P
15.00	70.00	1.50	10.00	Q
15.00	70.00	1.50	30.00	R
15.00	70.00	5.00	10.00	S
15.00	77.11	3.25	20.00	T

Equipment: Osmometer (Vitech 3300 - Germany) PH meter (PH Metrohm-Switzerland)
Turbiditimeter (Turbidimeter HACH - America), Platometer (Brix ANTONPAAR - Germany)
Colorimeter (Rang AVM - Germany).

Samples preparation: the solutions were prepared in different ratios according to RSM method so that, malt extract with brix 12 was thrown in sterilized containers and then, its brix was adjusted in the range of 7.5-8.5 using sterilized water and sugar. At the next step, the prepared beverage was divide in 300 cc containers, and 45 mg vitamin C was added to the all treatments and then, the treatments were formulated by adding calcium chloride, sodium chloride and magnesium chloride with amounts determined in Table 1. Each test was conducted in three replications.

Methodology

The research was conducted within two stages of chemical experiments and sensory test: at the first stage, chemical tests including pH, acidity, osmotic pressure, density, brix, turbidity and color under laboratory conditions were conducted according to Iranian Standard No2279 to evaluate physicochemical traits of isotonic beer and to conform to the chemical characteristics of beer and sports drink. By investigating the results of test and comparison to the standards of sports drinks and beer, the treatments out of standard range were eliminated. At the second stage of chemical tests according to the increase of turbidity over the time, the amount of turbidity was studied using Forcing test according to [13] and the treatments out of the standard range were eliminated. Sensory evaluation was carried out using triangular test[13] to evaluate sensory traits including taste.

Determination of osmotic pressure

Osmotic pressure was done using osmometer [21].

Determination of pH

pH measurement was conducted using pH meter [12].

Determination of turbidity

Turbidity measurement was done using turbiditimeter and through the following equation. The base of this device is light radiation from a source and measurement of the amount of light which are reflected by particles in water. Common units of turbidity include: NTU, FTU, EBC and ASBC [11].

$E.B.C = \frac{NTU}{4}$ Equation 1

Determination of color

Color was stated by EBS using colorimeter [4].

Determination of acidity

Acidity measurement was conducted by titration method and using the following equation:

$$A = \frac{0.009 \times v2 \times 100}{v1} \qquad \text{Equation 2}$$

Where:

V_2 is 0.1 normal sodium hydroxide by mm

V_1 is sample volume by cc

A is total acidity by gr/100cc

(Anonymous, 2009).

Determination of brix

Platometer was used to measure brix [4].

Forcing test

This test states the stability of various drink products in which the stability of the product is determined by measurement of some qualitative traits such as turbidity, etc. in this test a certain number of battles containing the product were placed in an incubator under 53^0 C. being under this temperature for 12 hours and immediately being placed under 2 ^{0}C temperature indicates stability of the product for a month, and being under 53 ^{0}C temperature for 24 hours and immediately being placed under 2 ^{0}C temperature indicates stability of the product for two month, and this trend can continue accordingly [4].

Sensory evaluation

Sensory evaluation of produced treatments was done using triangular test [4].

Data analysis method

Data analysis was conducted using RSM. Independent variables and responses are as following:

Independent variables: sodium chloride with five levels 17.89, 25, 47.50, 70 and 77.11 mg/100cc was used in the produced treatments formulations. Magnesium chloride with five levels 0.95, 1.50, 3.25, 5 and 5.55 mg/100cc was used in the produced treatments formulations. Calcium chloride with five levels 6.86, 10, 20, 30 and 33.16 mg/100cc was used in the produced treatments formulations.

Responses: turbidity measurement, measurement of osmotic pressure, pH measurement, acidity measurement, color measurement, brix measurement, density measurement.

RESULTS

Data analysis was conducted using response level and various tests. Regression coefficients were estimated and variance analysis table was drawn for each response (acidity, turbidity, osmotic pressure, total density, pH, plato and color).

Table 2-Analysis of variance pH, turbidity, osmosis, pH, total density Sports Beer

mu		**Haze**		**Acidity**		**df**	**Source**
P-Valu	Coefficient	P-Valu	Coefficient	P-Valu	Coefficient		Model
0.0011	318.20530	0.0001	-11.88318	0.0001	0.21002	9	Model
0.0019	+2.14848	0.0024	+2.28194	0.0614	0.50525×10^{-4}	1	X_1
0.0009	-14.6206	0.0001	-2.82469	0.0833	-2.2736×10^{-3}	1	X_2
0.0312	+0.40627	0.0001	+0.097075	0.0002	-1.16543×10^{-3}	1	X_3
0.3026	-0.026558	0.0005	-0.021235	0.0278	-1.11845×10^{-5}	1	X_1^2
0.0209	+2.18417	0.001	+0.63735	0.0345	$+8.00029 \times 10^{-4}$	1	X_2^2
0.0147	-0.014208	0.0111	$+2.59670 \times 10^3$	0.2902	$+5.08871 \times 10^{-6}$	1	X_3^2
0.0083	-0.42737	0.0001	-0.18791	0.001	-2.43800×10^{-4}	1	$X_1 X_2$
0.0454	+0.023152	0.0001	+0.014510	0.0001	$+3.42272 \times 10^{-5}$	1	$X_1 X_3$
0.1844	+0.082574	0.0482	-0.022561	0.0320	-1.11845×10^{-5}	1	$X_2 X_3$
0.3424	-	0.2058	-	0.7450	-	5	

0.05 Significant $_v$<

Na : MgX_3 : CaX_2: X_1

0.05 Significant p $_v$<

Table 3-Analysis of variance pH, turbidity, osmosis, pH, total density Sports Beer

PH		**Density**		**df**	**Source**
P-Valu	Coefficient	P-Valu	Coefficient		Model
0.0001	+1.02935	0.0001	+4.01899	9	Model
0.0001	$+1.97650 \times 10^{-4}$	0.0003	-3.80412×10^{-3}	1	X_1
0.0877	-6.21738×10^{-4}	0.0001	-0.010250	1	X_2
0.0004	$+7.23063 \times 10^{-5}$	0.1768	-1.39898×10^{-3}	1	X_3
0.4319	-4.81439×10^{-6}	0.0006	-2.51835×10^{-5}	1	X_1^2
0.3037	$+1.58156 \times 10^{-4}$	0.0006	$+1.08739 \times 10^{-3}$	1	X_2^2

0.2873	-1.04979×10^{-6}	0.0003	$+6.81855 \times 10^{-6}$	1	X_3^2
0.9640	-3.94180×10^{-4}	0.0001	$+5.72147 \times 10^{-6}$	1	X_1X_2
0.0046	$+1.72761 \times 10^{-6}$	0.0016	$+4.620092 \times 10^{-5}$	1	X_1X_3
0.1352	-6.81383×10^{-7}	0.7743	$+1.18306 \times 10^{-4}$	1	X_2X_3
0.2590	-	0.2396	-	5	

Na : MgX_3 : CaX_2: X_1

According to Table 2, 3 it is seen that, about acidity, linear effect of sodium (X_3) and interaction between calcium and magnesium (X_1, X_2), calcium and sodium (X_1, X_3), magnesium and sodium (X_2, X_3) and also, non-linear effect of calcium (X_1^2) and magnesium (X_2^2) are significant ($P<0.05$) and other effects are not significant ($P>0.05$).

About turbidity, linear effects of calcium (X_1), magnesium (X_2), sodium (X_3) and interaction between calcium and magnesium (X_1, X_2), calcium and sodium (X_1, X_3), magnesium and sodium (X_2, X_3) and also, non-linear effect of calcium (X_1^2), magnesium (X_2^2) and sodium (X_3^2)are significant ($P<0.05$).

About osmotic pressure, linear effect of calcium (X_1), magnesium (X_2), sodium (X_3) and interaction between calcium and magnesium (X_1, X_2), calcium and sodium (X_1, X_3) and also, non-linear effect of magnesium (X_2^2) and sodium (X_3^2) are significant ($P<0.05$) and other effects are not significant ($P>0.05$).

For pH, linear effect of calcium (X_1), magnesium (X_2), sodium (X_3) and interaction between calcium and sodium (X_1, X_3) are significant ($P<0.05$) and other effects are not significant ($P>0.05$).

About density, linear effect of magnesium (X_2), sodium (X_3) and interaction between calcium and magnesium (X_1, X_2), calcium and sodium (X_1, X_3) and also, non-linear effect of calcium (X_1^2), magnesium (X_2^2) and sodium (X_3^2) are significant ($P<0.05$) and the other effects are not significant ($P>0.05$).

Table 4-Summaryof results of statistical models defined acidity, PH, turbidity, density osmosis ,beerand sports

Density	**mu**	**Haze**	PH	**Acidity**	Variable
0.9645	0.8871	0.9711	0.9435	0.9373	R^2
0.9325	0.7855	0.9451	0.8927	0.8809	$R^2_{adjusted}$
0.7957	0.3439	0.8206	0.6856	0.7003	$R^2_{Predicted}$

R^2: Determination coefficient shows that, how much the defined model states the variations of variable y.

$R^2_{adjusted}$: states R^2 which has been defined for the number of existing factors in corrected model.

$R^2_{Predicted}$: predicts existing factors in the model.

According to Table 4, high amounts of $R^2$and partial difference of $R^2_{adjusted}$and $R^2_{Predicted}$ states high precision of the model in prediction.

Investigation of the results achieved from color and plato test

Since the results of color and plato test were a constant value (color: 12 EBC and brix: 8.6) in all treatments, therefore, variance of this characteristic was zero and there was no possibility for variance analysis.

Optimization

Table 5-Results ofthe optimization

Percentcompliance	**mu** mos/lit()	(NTU)**Haze**	**Na** (mg/100ml)	**Mg** (mg/100ml)	**Ca** (mg/100ml)	**Number**
96.4	304.223	3.85461	25.00	5.00	10.00	1
96.4	304.355	3.85987	25.00	4.98	10.00	2
96.4	304.437	3.86318	25.00	4.97	10.00	3
96.3	304.812	3.87833	25.00	4.91	10.00	4
96.2	305.114	3.89056	25.00	4.86	10.00	5
95.1	305.309	3.89861	25.00	4.83	10.00	6
94.7	309.819	4.08098	25.00	4.11	10.00	7
94.2	306.085	4.13847	25.00	5.00	12.62	8
93.1	306.761	4.22064	25.00	4.90	12.71	9
96.2	307.812	4.40156	25.00	5.00	15.04	10

After fitting the models associated with the evaluated traits, optimization was conducted considering the effective factors in sports beer including osmotic pressure, at the range of 240-330 mos/lit, maximum turbidity 8NTU. According to the results of Table 5, it can be seen that, calcium by 10 mg/100cc, magnesium by 5 mg/100ml and sodium by 25 mg/100ml can make the best conditions. In such conditions, the optimal turbidity is 3.85461 NTU and osmotic pressure is 304.223 mos/lit. This matter shows that, by optimization, it is possible to produce a formulation with 96.4% utility.

Investigation of the results achieved from turbidity test

Achieved results from turbidity test have been shown in Graph (1). By means comparison it was found that, there is a significant difference among the treatments. Consequently, eight treatments including S, Q, J, G, F, E, B, A were confirmed in terms of turbidity due to being placed in the standard turbidity range.

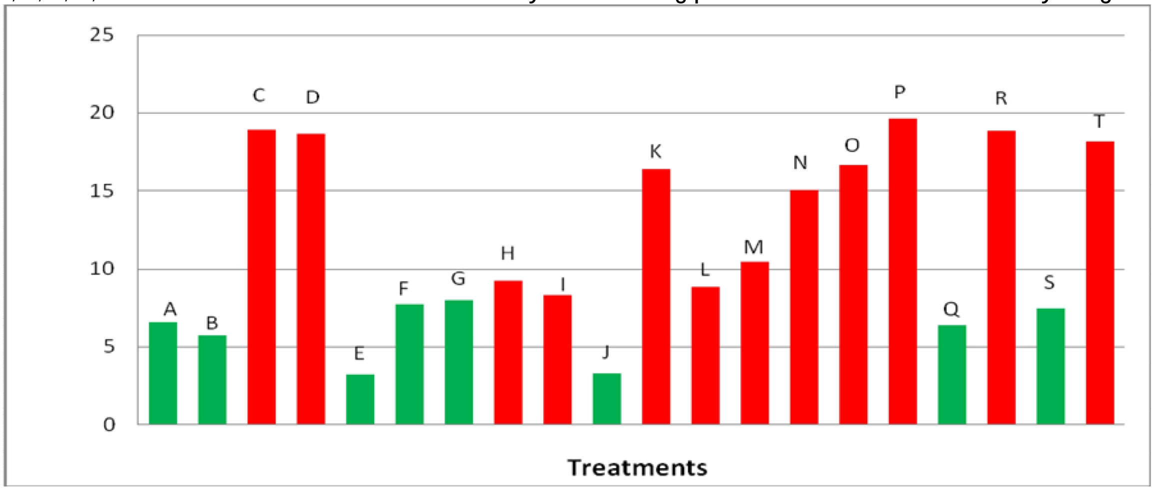

Figure 1. Means comparison of turbidity in sports beer treatments
Turbidity out of the standard range
- Turbidity within the standard range

Investigation of the results achieved from osmotic pressure test

The results of osmotic pressure in Graph(2) have been shown. By means comparison it was found that, there is a significant difference among the treatments. Consequently, the treatments including S, R, N, L, K, J, I, H, G, F, E, C, B, A were confirmed in terms of turbidity due to being placed in the standard osmotic pressure range.

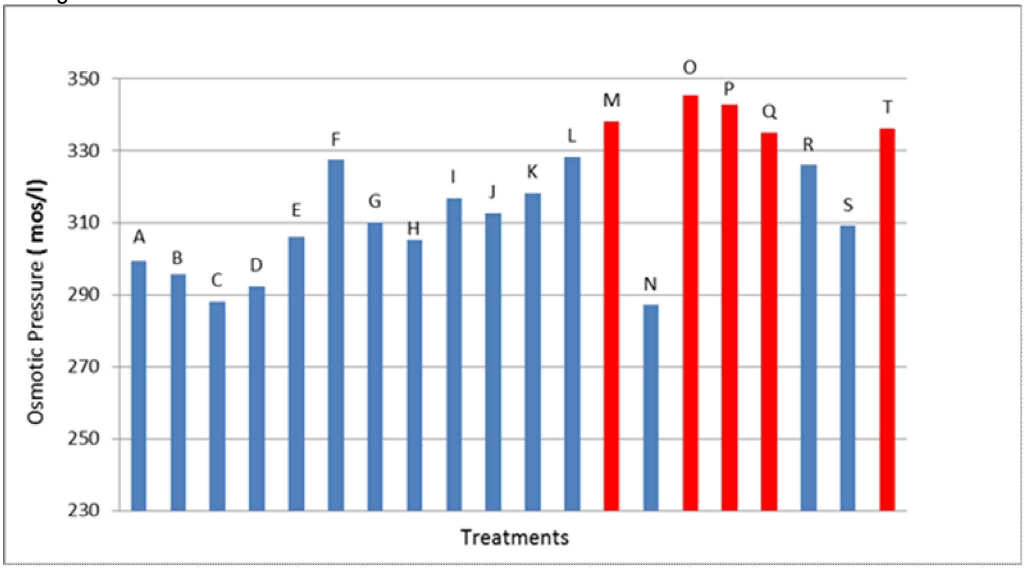

Figure 2. Means comparison of osmotic pressure in sports beer treatments
Turbidity out of the standard range
- Turbidity within the standard range

Investigation of the results achieved from acidity, pH, density, color and brix

It is required to be mentioned that, adding solute causes to change the other physicochemical parameter including acidity, pH, density, color and plato, but they were not out ofthe determined range in the Iranian Standard No 2279.

Finally, considering the elimination of treatments having turbidity and osmotic pressure out of the standard limit, only 7 treatments (A, B, E, F, G, J, S) had proper both turbidity and osmotic pressure. Since turbidity is changeable over the time, the amount of turbidity was investigated in the selected 7 treatments by Forcing test.

Investigation of the results achieved from Forcing test

Among the 7 selected treatments in quality control tests, three treatments (B, E, J) had an appropriate stability during 60 days.

Investigation of the results achieved from turbidity graphs in Forcing test

In all treatments, turbidity was increased over the time during 60 days. In treatments A, F, G, S, since the beginning, in treatment A within the first 30 days, the turbidity became out of the standard range.

Investigation of the results achieved from triangular test

According to statistical results of the characteristic taste, there was a significant difference among treatments B, E, J at confidence level of 0.05 ($P<0.05$). According to statistical results of the characteristic bitterness there was no significant difference among treatments B, E, J ($P>0.05$). According to statistical results of the characteristic salinity, there was a significant difference among treatments B, E, J ($P<0.05$). Totally, treatment B had the highest rate and treatment J had the lowest rate in comparison of bitterness and salinity.

Discussion

The effect of independent variables on acidity and pH of sports beer

By increasing of each cation, acidity was increased which can be stated that, magnesium, calcium and sodium react to alkalinity factors existing in the beer such as carbonate, bicarbonate and hydroxide and affect the amount of acidity and pH; so that, in presence of each cations, the reactions go toward alkalinity factors reduction which causes to increase acidity and pH reduction. In a book entitled water in the process of malt and beer production, Moll has explained this process in 1979. Biescas et al[3] mentioned that, pH is changed when the additives are added to the primary material. Adding salts containing Ca, Na, etc. ions will affect acidity and pH due to creation of acidic and buffer compounds in the solution.

Fischer et al[8]found that, adding calcium by 0.005 to 0.015 gr to sports drink can cause to increase acidity and pH reduction due to creation of complex between calcium and phosphate.

The effect of independent variables on the amount of osmotic pressure of sports beer

The ions calcium, magnesium and sodium cause to increase osmotic pressure. This matter according to Kelly et al[15] is due to the increase of electrical conductivity by increasing of dissolved ions and considering the linear relationship between electrical conductivity and osmotic pressure ($OP=0.36EC$), osmotic pressure also would be increased. By the way, osmotic pressure has a direct relation to the concentration of dissolved material ($OP=MRT$) and is independent from the nature of dissolved material. So, increase of osmotic pressure caused by adding calcium, magnesium and sodium is justifiable.

OP: osmotic pressure, M: concentration of dissolved material, R: universal gas constant, T: temperature

Also, similar results were obtained in investigating the effect of mineral solute on osmotic pressure by Biescas et al[3] They stated that, according to the equation Osmolality= ϕnc, creation of osmotic pressure in the solvent by each mole of the added ions, adding mineral solute causes to increase osmotic pressure. When magnesium chloride was used by 15 mg/l, osmotic pressure was 111 mosm/kg which was increased by increasing magnesium chloride by 30 mg/l, u to 220 mosm/g.

ϕ:osmotic coefficient, n: the number of molecules components which may be separated, c: molar concentration

The effect of independent variables on the amount of sports beer turbidity

All the three ions including calcium, magnesium and sodium cause to increase turbidity of which the effect of calcium and magnesium was higher. By the way, sodium ion has no significant impact on turbidity increase lonely, and its impact can be due to being along with calcium and magnesium ions. Comric[7] stated that, calcium ion can react with the existing phosphate in the beer and create sediment. Also, calcium can be combined to the oxalate existing in malt which causes to increase turbidity. Magnesium also makes some reactions such as calcium but, magnesium salt has more breakup and consequently, it increases turbidity less than calcium. Electromagnetic forces between opposite ions cause covalent and ionic bonds. In beer also, the added materials are ionized due to breakup and absorb dissolved ions and ultimately cause turbidity among the particles and the liquid around. Due to the lack of appropriateness of high turbidity in beer and the impact of the three added ions in turbidity increase, determination of a suitable formulation is much important.

Hutt et al[12] conducted a study on isotonic drink production for 6-12 years old children and used juice as the primary material and some solutes such as calcium, sodium and potassium and vitamin C. The results showed that, adding calcium, sodium and potassium will cause to increase turbidity due to forming dissolved salts and bulky complexes which is consistent to the results of the present study.

The effect of independent variables on the amount of sports beer turbidity

All the three ions including calcium, magnesium and sodium cause to increase density while, magnesium had a higher impact on density. This matter is due to the increase of molecular mass caused by the increase of ions and consequently increasing density.

[13] conducted a study on comparison of physical traits including density, viscosity, refractive index and electrical conductivity of two-component solution of sodium chloride with concentration of 10-20 weight percentage, in two types of soft water and hard water at 25 °C. The results showed that, the mentioned physical traits has an ascending trend by increasing the concentration of sodium chloride from 10 to 20%.

Also, the increase of physical traits in the solution containing soft water-sodium chloride than for hard water-sodium chloride.

The effect of independent variables on the amount of sports beer color

Considering that, color in beer is resulted from the use of malt, and the used mineral solutes were white or colorless, and the treatments' color was denser than the mineral solutes, no impact was observed and considering that, the results of color test were constant in all the treatments (12 EBC), therefore, the variance of this trait was zero and there was no possibility for variance analysis.

Savel et al[22] conducted a study on the factors creating the color in beer and concluded that, sugar caramelization and anaerobic reactions were the only factors creating the color in beer, and the beer color is decreased by oxidation. They showed that, adding mineral solutes makes no change in the beer color.

Collin and Callemin (2007) showed that, in addition to Millard reaction, rearrangement of monomer Flavan-3-ol structure causes to change the color of beer, and achieved similar results with the resent study about the lack of changeability of beer color by adding mineral solute.

The effect of independent variables on the amount of sports beer brix

Considering that, beer brix is mostly affected by the use of sugar and malt, and adding solutes has been done after processing, no change was observed on the amount of brix and according to the constant (8.6) results of brix test in all tested treatments, therefore, variance of this trait was zero and variance analysis was not possible.

Briceeno[5] investigated the variations of sports drink brix by adding carbohydrate including sugar by 50 to 150 gr/l and showed that, increase of sodium chloride by 0.005 to 0.02 gr/l had no impact on the amount of brix and only by the increase of sugar, the drink brix was increased.

Investigation of the results achieved from laboratory tests on the treatments

Results of turbidity test showed that, by increasing the amount of mineral solute, turbidity also will be increased. There was a significant difference ($P<0.05$) among treatments (A, B, Q), (C, D, P, R). (E, J), (F, G, H, I, L, S), (K, O, T), (N, M).Considering the significance of the interaction of minerals including calcium chloride, magnesium chloride and sodium chloride and turbidity, and with regard to the presence of mineral solute with various amounts in the treatments formulation, this matter will be justifiable. 12 treatments (C, D, H, I, , L, M, N, O, P, R) were eliminated due to having turbidity out of the standard range and 8 treatments (A, B, E, F, G, J, Q) were selected due to having standard turbidity.

The results of osmotic pressure showed that, by changing the amount of mineral solute, osmotic pressure also is increased. As it has been shown in the graph, mean osmotic pressure had a significant difference among the treatments at the probability level of 5%. Considering the significance of the interaction of calcium chloride, magnesium chloride and sodium chloride on osmotic and with regard to the presence of mineral materials with different amounts in the treatments formulations, this matter is justifiable. According to [14] and the determined range for osmotic pressure (290 ± %15 mos/l), five treatments (M,O,P,Q,T) were eliminated and 15 treatments (A,B,C,D,E,F,G,H,I,J,K,L,N) were selected due to having appropriate osmotic pressure.

Investigation of the results achieved from Forcing test

According to the results of Forcing test, turbidity has been increased over the time which is due to creation of bulky complexes resulted from polyphenols and proteins existing in the beer which cause to make and increase turbidity over the time.

Hosseini et al[11] conducted a study on physicochemical traits and beer stability during 6 months maintenance. The results indicated that, physicochemical traits were changed over the time of which turbidity was the most important. They considered the reaction between polyphenols and proteins forming the large complexes as the cause of turbidity increase.

Investigation of the results achieved from sensory test

The results of sensory test of flavor and taste from treatments B, E, J showed a significant difference ($p<0.05$) among all the treatments and there was no significant difference for bitterness trait ($p>0.05$).

Goldammer [10] in a topic of additive mineral salts has mentioned that, calcium sulfate makes a bitter taste due to existence of calcium ion.

Investigating the accuracy of the model prediction used in beer formulation

According to the results of beer formulation optimization and considering the effective traits in sports beer including osmotic pressure at the range of 240-330 mos/lit, maximum turbidity by 2 EBC or 8 NTU and considering the results of Table (3), calcium by 10 ml/100 cc, magnesium by 5 mg/100 ml and sodium by 25 mg/100 ml can provide a product with optimal conditions. This formulation was consistent to the formulation of selected treatment (B) after physicochemical, Forcing test and triangular test. It showed that, using optimization, a formulation can be produced which is able to provide desirable turbidity and osmotic pressure. These results represent high matching power of the model in predicting and optimizing the drink formulation.

CONCLUSION

This research was conducted by chemical test and sensory test. At the first stage, chemical test was carried out to investigate the effect of calcium chloride (with five concentrations: 6.86, 10, 20, 30, 33, 33.16 mg/100cc), magnesium chloride (with five concentrations: 0.95, 1.50, 3.25, 5, 5.55 mg/100 cc) and sodium chloride (with five concentrations: 17.89, 25, 47.50, 70, 77.11 mg/100 cc) on physicochemical traits of isotonic beer and also, Forcing test was done to evaluate increase of turbidity during 60 days, and ultimately, sensory test was conducted in all the treatments. According to the results of chemical test, by increasing of each solute, physicochemical traits including acidity, turbidity, osmotic pressure and density were increased and pH was decreased and these solute had no impact on color and plato.

Also, the results of experiments showed that, the traits acidity, density, pH, color and plato of all treatments were within the standard range. Considering the importance of turbidity and osmotic pressure in beer sports drinks, some treatments (C,D,H,I,K,L,M,N,O,P,Q,R,T) were eliminated due to being out of standard range in terms of osmotic pressure and turbidity, and 7 treatments(A,B,E,F,G,J,S) were selected.

Also considering the increase of turbidity over the time, Forcing test was conducted at the second stage of chemical tests, and ultimately, treatments B, E, J had appropriate turbidity.

According to the results of sensory test of isotonic beer, there was a significant difference ($P<0.05$) among the treatments for two traits including salinity and flavor, and the highest rate was for treatment B with formulation: Ca: 10 mg/100cc, Mg: 5mg/100cc, Na: 25 mg/100cc, and the lowest rate was for treatment J with formulation: Ca: 6.84 mg/100cc, Mg: 3.25 mg/100cc, Na: 47.5 mg/100cc. According to the results of chemical tests, sensory tests and optimization, treatment B was selected as the best treatment.

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