Enhance of Flavor and Some Microbial, Physiochemical and Sensory Properties of Yogurt by Threonine and Glycine

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Abstract
This study was aimed to enhance the flavor and some microbial, physiochemical and sensorial properties of yogurt by threonine and glycine. This study included the estimation (acetaldehyde, acetone and ethanol), acetaldehyde content was 13.46, 13.9, 19.52, 13.99, 28.76, 27.31 and 18.52 mg L-1 for (A) control treatment, (B) glycine 1g /L , (C) threonine 1 g/ L, (D) glycine 3 g/ L, (E) threonine 3 g/ L, (F) threonine 3g + glycine 1g / L and (G) glycine 3g + threonine 1g / L treatments respectively. Acetone content was 8.38, 8.26, 5.71, 8.12, 3.84, 3.11 and 5.49 mg L-1 for A, B, C, D, E, F and G treatments respectively. Ethanol content was 10.25, 10.17, 6.39, 10.16, 4.56, 4.16 and 6.21 mg L-1 for A, B, C, D, E, F and G treatments respectively. Lactobacillus bulgaricus count after the first day of manufacture were 7.38, 7.34, 7.49, 7.39, 7.70, 7.68 and 7.50 cfu mL-1 for A, B, C, D, E, F and G treatments respectively, after 28 days of manufacture was 6.25, 6.23, 6.32, 6.27, 6.51, 6.47 and 6.38 cfu mL-1 for A, B, C, D, E, F and G treatments respectively. Streptococcus thermophilus count after the first day of manufacture were 9.20, 9.14, 9.38, 9.30, 10.62, 10.53 and 9.47 cfu mL-1 for A, B, C, D, E, F and G treatments respectively, after 28 days of manufacture were 8.25, 8.11, 8.34, 8.27, 9.17, 9.11 and 8.36 cfu mL-1 for A, B, C, D, E, F and G treatments respectively after 28 days. The chemical composition did not change, there were no significant differences compared with treatments. Sensory evaluation score showed significant differences ($P \leq 0.05$) among the treatments.

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Introduction

Yogurt has topped the list of fermented dairy thousands of years ago and has entered the human diet, it becomes one of the main meals that provide him with the important nutrients the body needs in performing its biological activities. Yogurt was found by chance as a result of the activity of microorganisms and their effects on the components of milk, this coincidence was an important factor in prolonging the shelf life of milk by turning the milk into fermented milk that lasts for several days, the most important thing that distinguishes yogurt from other fermented milk products is the distinctive flavor of yogurt, which is due to lactic acid and the rest of the flavor compounds resulting from the fermentation processes of milk by the yogurt starter, among the most prominent of these compounds are aldehydes, ketones, amino acids and alcohols (Dan *et al.*, 2017).

Acetaldehyde is one of the most important flavor compounds in yogurt which is an important criterion for the acceptance of the product by consumers. Glucose, catechol, glyceraldehyde, acetylene, and amino acids such as threonine and deoxyribonucleic acid (DNA) can act as the acetaldehyde precursors, it was found that the weak flavor in the yogurt product when the acetaldehyde level was less than 4.0 ppm, which is considered an imperfect amount, while the good flavor of the yogurt that produced acetaldehyde level reaches 8.0 ppm or more (Routray and

Mishra, 2011). Alcoholic flavor compounds such as ethanol and acetone, they are other necessary compounds in yogurt, but their presence is within certain limits and increasing their production is undesirable (Rul, 2017).

Amino acids (threonine and glycine) are one of the most important sources of nutrition for the yogurt starter bacteria in order to complete the processes of growth, reproduction and production, threonine is one of the most important sources of flavor compounds for the yogurt product (Beshkova *et al.*, 1998). The study was aimed to improve the flavor and some microbial, physicochemical and sensorial properties of yogurt by threonine and glycine.

Materials and Methods

Amino acids were used (Hi-media, India). Yogurt was produced using a standard yogurt manufacturing process (Tamime and Robinson, 2007). The following treatments were used (A) Control treatment , (B) glycine 1g/L, (C) threonine 1 g/ L, (D) glycine 3 g/L , (E) threonine 3 g/L , (F) threonine 3g + glycine 1g/L , (G) glycine 3g + threonine 1g/L. Yogurt starter bacteria (Sacco Lyofast company, Italy) containing *Lb. bulgaricus* and *S. thermophilus* was used in manufacturing. Yogurt starter bacteria was activated individually in MRS broth and M17 broth (Hi-media, India), (Savini, 2010). To determine of starter count; MRS agar was used for *Lb. bulgaricus* count and incubated at 45 ◦C for 72 hours. M17 agar was used for *S. thermophilus* count (incubated at 37◦C for 48 h). MacConkey agar was used for coliform bacteria count (incubated at 37◦C for 48 h). Nutrient agar was used for psycrotrophic bacteria count (incubated at 4◦C for 10 days). PDA agar was used for molds and yeasts count (incubated at 25°C for 5 days), (APHA, 1978). Moisture, protein, fat, ash and pH were determined according to A.O.A.C. (2016). Flavor compounds (acetaldehyde, acetone and ethanol) were evaluated by gas chromatography, according to A.O.A.C. (2016). Sensory evaluation was performed by using the evaluation form for the yogurt according to Nelson and Trout (1964). The statistical analysis system SAS-CRD (2012) program was used to detect the effect of different factors on study parameters.

Results and Discussion

Yogurt starter bacteria

The results in (table 1) showed *Lb. bulgaricus* count in yogurt treatments at refrigerated storage. The count after the first day were 7.38, 7.34, 7.49, 7.39, 7.70, 7.68 and 7.50 *cfu* mL^{-1} for A, B, C, D, E, F and G treatments respectively, then gradual decreasing through storage periods to registered after 28 days 6.25, 6.23, 6.32, 6.27, 6.51, 6.47, 6.38 *cfu* mL^{-1} respectively. *S. thermophilus* count were determined after the first day 9.20, 9.14, 9.38, 9.30, 10.62, 10.53, 9.47 *cfu* mL^{-1} for A, B, C, D, E, F and G respectively, then gradual decreasing through storage periods to registered after 28 days 8.25, 8.11, 8.34, 8.27, 9.17, 9.11, 8.36 *cfu* mL^{-1} respectively. Decreasing *Lb. bulgaricus* and S. *thermophilus* count may be due to dropping in pH, high acidity was due to the consumption of lactose and the production of lactic acid, and the change in environmental conditions their factors affected the viability of cells gradually during storage, the other reason may be due to difference of osmosis pressure of the medium, an increase in secondary metabolites in the environment, shrinkage of the clot. Treatment E (threonine 3g/L) was the highest treatment in *S. thermophilus* and *Lb. bulgaricus* count because threonine good nutrient that promoted the growth and activity of yogurt starter bacteria, threonine is one of the most important sources of nitrogen stimulating growth and reproduction, while it was the lowest value for (B) glycine 1g/L, the reason for this may be attributed to the fact that some strains of *S. thermophilus* have weak growth in the presence of glycine, these results are in agreement with (Tamime and Robinson, 2007; Mega *et al.*, 2019).

Coliform, psycrotrophic bacteria and yeastand mold

Coliform, psycrotrophic bacteria yeast and mold were absent from A, B, C, D, E, F and G treatments of yogurt (table 1), this may be attributed to the selection of good quality raw materials that have been used in yogurt manufacture, are essential in controlling microbial contaminations in food, the efficiency of the pasteurization process and the absence of the manufacturing and storage from the causes of contamination with pathogenic and spoilage microorganisms causes spoilage of yogurt, addition to the activity of yogurt starter bacteria in the production of lactic acid, acetic acid and bacteriocins, which acts as an antibacterial and antifungal, these results are in agreement with the findings (Warakaulle *et al.*, 2014; Matter *et al.*, 2016)

Table 1. Microbiological results of yogurt treated with threonine and glycine during refrigerated storage (28day/4°C)

Treatments	1 DAY	7 DAY	14 DAY	21 DAY	28 DAY	LSD value
***Lb. bulgaricus* (*CFU* mL^{-1})**						
A	7.38	6.49	6.34	6.30	6.25	**0.892 ***
B	7.34	6.44	6.30	6.27	6.23	**0.844 ***
C	7.49	6.51	6.39	6.36	6.32	**1.02 ***
D	7.39	6.49	6.36	6.32	6.27	**0.893 ***
E	7.70	6.81	6.74	6.64	6.51	**1.16 ***
F	7.68	6.78	6.69	6.57	6.47	**0.906 ***
G	7.50	6.51	6.44	6.41	6.38	**1.14 ***
LSD value	**0.693 NS**	**0.588 NS**	**0.702 NS**	**0.578 NS**	**0.598 NS**	---
***S. thermophillus* (*CFU* mL^{-1})**						
A	9.20	8.56	8.46	8.39	8.25	**0.803 ***
B	9.14	8.49	8.39	8.30	8.11	**0.895 ***
C	9.38	8.63	8.53	8.46	8.34	**0.784 ***
D	9.30	8.59	8.49	8.41	8.27	**0.892 ***
E	10.62	9.62	9.47	9.38	9.17	**1.25***
F	10.53	9.59	9.44	9.34	9.11	**0.894 ***
G	9.47	8.65	8.56	8.47	8.36	**1.05 ***
LSD value	**1.07 ***	**1.14 ***	**1.08 ***	**0.893 ***	**0.892 ***	---
Coliform bacteria (*CFU* mL^{-1})						
A	Nil	Nil	Nil	Nil	Nil	**NS**
B	Nil	Nil	Nil	Nil	Nil	**NS**
C	Nil	Nil	Nil	Nil	Nil	**NS**
D	Nil	Nil	Nil	Nil	Nil	**NS**
E	Nil	Nil	Nil	Nil	Nil	**NS**
F	Nil	Nil	Nil	Nil	Nil	**NS**
G	Nil	Nil	Nil	Nil	Nil	**NS**
LSD value	**NS**	**NS**	**NS**	**NS**	**NS**	---

Psycrotrophic bacteria (*CFU* mL^{-1})						
A	Nil	Nil	Nil	Nil	Nil	**NS**
B	Nil	Nil	Nil	Nil	Nil	**NS**
C	Nil	Nil	Nil	Nil	Nil	**NS**
D	Nil	Nil	Nil	Nil	Nil	**NS**
E	Nil	Nil	Nil	Nil	Nil	**NS**
F	Nil	Nil	Nil	Nil	Nil	**NS**
G	Nil	Nil	Nil	Nil	Nil	**NS**
LSD value	**NS**	**NS**	**NS**	**NS**	**NS**	**---**
Yeast and Mold (*CFU* mL^{-1})						
A	Nil	Nil	Nil	Nil	Nil	**NS**
B	Nil	Nil	Nil	Nil	Nil	**NS**
C	Nil	Nil	Nil	Nil	Nil	**NS**
D	Nil	Nil	Nil	Nil	Nil	**NS**
E	Nil	Nil	Nil	Nil	Nil	**NS**
F	Nil	Nil	Nil	Nil	Nil	**NS**
G	Nil	Nil	Nil	Nil	Nil	**NS**
LSD value	**NS**	**NS**	**NS**	**NS**	**NS**	**---**
*** (P≤0.05), NS: Non-Significant**.						

***(A) Control, (B) Glycine 1g/L, (C)Threonine 1g/L, (D) Glycine 3g/L, (E) Threonine 3g/L, (F) Threonine 3g+Glycine 1g/L, (G) Glycine 3g+threonine 1g/L**

Physiochemical properties

The results are shown in the (table 2), pH values on the first day after manufacturing were 4.42, 4.44, 4.45, 4.44, 4.47, 4.46 for A, B, C, D, E, F, and G treatments respectively, then gradually decreased after 28 days of refrigerated storage became 4.06, 4.1, 4.25, 4.14, 4.31, 4.28 and 4.24 respectively, the reason was yogurt starter bacteria consumed lactose and produced lactic acid, which led to a decrease pH values, these results are consistent with the results obtained by (Warakaulle *et al.*, 2014). There was a slight decrease in water during the storage period. The reason for this was the evaporation of moisture during storage which leads to an increase in protein, fat and ash, this is consistent with what he found (Qureshi *et al.*, 2011). Protein, fat and ash increased during the storage period and for treatments, including the control treatment, these results are consistent with the results obtained by (Hassan and Imran, 2010), which indicated that the contents increased during the storage period. These results are consistent with the results obtained by (Qureshi *et al.*, 2011).

Table2. Physiochemical properties results of yogurt treated with threonine and glycine during refrigerated storage (28 day/4°C)

Treatments	1 DAY	7 DAY	14 DAY	21 DAY	28 DAY	LSD value
pH						
A	4.42	4.4	4.37	4.2	4.06	**0.537 NS**
B	4.44	4.42	4.38	4.24	4.1	**0.483 NS**
C	4.45	4.43	4.41	4.33	4.25	**0.477 NS**
D	4.44	4.42	4.39	4.25	4.14	**0.492 NS**
E	4.47	4.46	4.44	4.38	4.31	**0.398 NS**
F	4.46	4.45	4.42	4.36	4.28	**0.398 NS**

G	4.45	4.43	4.4	4.3	4.24	**0.378NS**
LSD value	**0.193 NS**	**0.187 NS**	**0.166 NS**	**0.205 NS**	**0.347 NS**	**---**
moisture %						
A	86.80	86.62	86.50	86.38	86.28	**2.41 NS**
B	86.72	86.63	86.49	86.35	86.17	**2.07 NS**
C	86.77	86.57	86.40	86.33	86.25	**1.98 NS**
D	86.54	86.36	86.30	86.21	86.08	**2.26 NS**
E	86.63	86.55	86.43	86.29	86.12	**2.55 NS**
F	86.69	86.58	86.47	86.31	86.1	**2.09 NS**
G	86.57	86.46	86.33	86.2	86.04	**2.26 NS**
LSD value	**2.58 NS**	**2.71 NS**	**2.44 NS**	**3.07 NS**	**3.71 NS**	**---**
Protein%						
A	3.05	3.11	3.2	3.25	3.32	**0.368 NS**
B	3.12	3.18	3.27	3.33	3.39	**0.308 NS**
C	3.08	3.13	3.19	3.27	3.34	**0.371 NS**
D	3.27	3.31	3.4	3.49	3.55	**0.302 NS**
E	3.19	3.25	3.34	3.41	3.45	**0.358 NS**
F	3.15	3.21	3.3	3.42	3.46	**0.387 NS**
G	3.29	3.36	3.44	3.52	3.59	**0.339 NS**
LSD value	**0.382 NS**	**0.307 NS**	**0.398 NS**	**0.355 NS**	**0.386 NS**	**--**
Fat%						
A	3.38	3.39	3.43	3.49	3.52	**0.376 NS**
B	3.37	3.37	3.41	3.49	3.51	**0.347 NS**
C	3.35	3.38	3.41	3.48	3.49	**0.327 NS**
D	3.36	3.38	3.42	3.48	3.5	**0.298 NS**
E	3.35	3.39	3.43	3.48	3.49	**0.218 NS**
F	3.35	3.37	3.42	3.49	3.5	**0.217 NS**
G	3.36	3.38	3.43	3.48	3.5	**0.205 NS**
LSD value	**0.216 NS**	**0.292 NS**	**0.218 NS**	**0.198 NS**	**0.302 NS**	**---**
Ash%						
A	0.69	0.7	0.74	0.75	0.76	**0.178 NS**
B	0.7	0.71	0.75	0.76	0.77	**0.207 NS**
C	0.69	0.7	0.74	0.75	0.76	**0.163 NS**
D	0.71	0.72	0.76	0.78	0.79	**0.166 NS**
E	0.69	0.7	0.74	0.75	0.77	**0.187 NS**
F	0.7	0.71	0.75	0.77	0.78	**0.108 NS**
G	0.72	0.73	0.77	0.79	0.8	**0.185 NS**
LSD value	**0.204 NS**	**0.144 NS**	**0.137 NS**	**0.152 NS**	**0.147 NS**	**---**
*** (P≤0.05), NS: Non-Significant.**						

***(A) Control, (B) Glycine 1g/L, (C)Threonine 1g/L, (D) Glycine 3g/L, (E) Threonine 3g/L, (F) Threonine 3g+Glycine 1g/L, (G) Glycine 3g+threonine 1g/L**

Acetaldehyde Content

The results are shown in the (figure 1), acetaldehyde values were 13.46, 13.9, 19.52, 13.99, 28.76, 27.31 and 18.52 (mg L^{-1}) in the treatments A, B, C, D, E, F and G respectively, it was found that acetaldehyde content increased in (E) threonine 3 g/ L and (F) threonine 3g + glycine 1g / L compared with the rest of the treatments because threonine represents one of the pathways of acetaldehyde formation by threonine aldolase (serine hydroxymethyl transferase), which converts threonine into acetaldehyde and releases glycine (Chaves *et al.*, 2002; Rul, 2017).

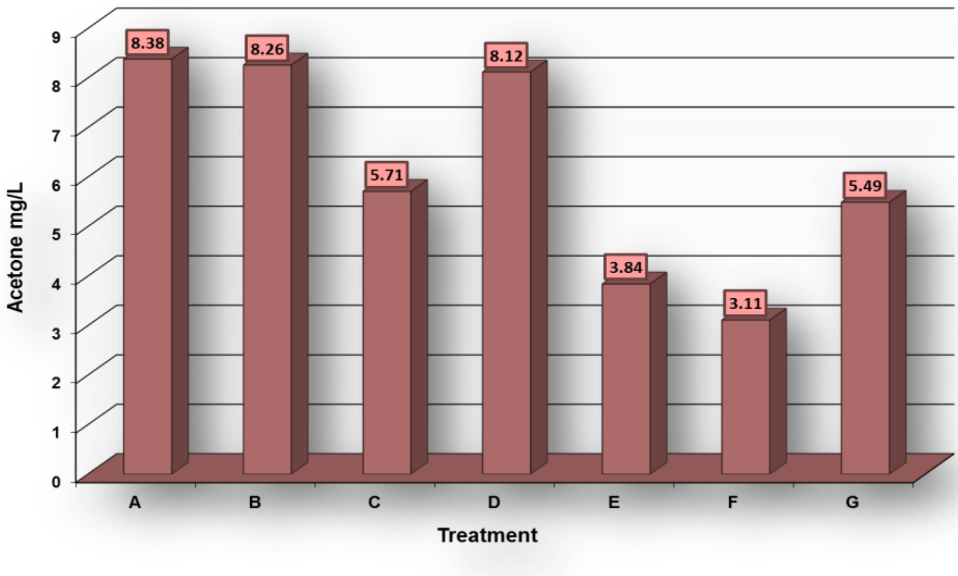

Figure 1. Acetaldehyde content (mg L^{-1}) in the yogurt samples experimental treated with threonine and glycine during refrigerated storage (7 day / 4°C) * (P≤0.05)

Acetone Content

By looking at the (figure 2), acetone values were 8.38, 8.26, 5.71, 8.12, 3.84, 3.11 and 5.49 (mg L^{-1}) for the treatments A, B, C, D, E, F and G respectively, it was found that acetone content decreased in (E) threonine 3 g/ L and (F) threonine 3g + glycine 1g / L compared with the rest of the treatments, the reason for this is that these treatments contain a high level of threonine, which is a good nutrient for the yogurt starter bacteria, which increases the activity of bacteria in the production of alcohol dehydrogenase was removed the hydrogen atom from alcohol and converts it to acetaldehyde as one of the pathways for the production of acetaldehyde from alcohol (Chaves *et al.*, 2002; Rul, 2017)

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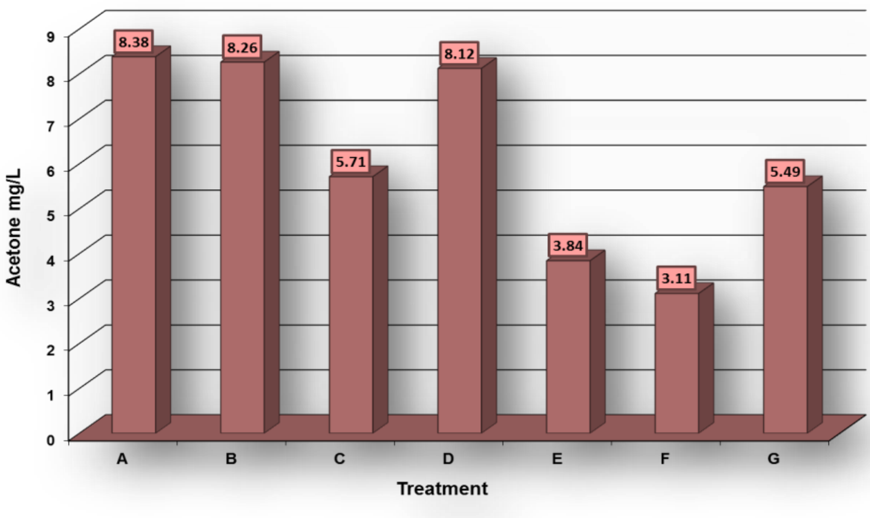

Figure 2. Acetone content (mg L-1) in yogurt samples experimental treated with threonine and glycine during refrigerated storage (7 day / 4°C) * (P≤0.05)

Ethanol Content

By looking at the (Figure 3), ethanol values were 10.25, 10.17, 6.39, 10.16, 4.56 , 4.16 and 6.21 (mg L^{-1}) for the treatments A, B, C, D, E, F and G respectively, it was found that acetone content decreased in (E) threonine 3 g/ L and (F) threonine 3g + glycine 1g / L compared with the rest of the treatments, the reason for this is that these treatments contain a high level of threonine, which is a good nutrient for the yogurt starter, which increases the activity of bacteria in the production of alcohol dehydrogenase was removed the hydrogen atom from ethanol and converts it to acetaldehyde as one of the pathways for the production of acetaldehyde from ethanol (Chaves *et al.*, 2002; Rul, 2017), reducing the percentage of ethanol to the limits of these values brings us many benefits, such as getting rid of the undesirable alcoholic taste in the yogurt product.

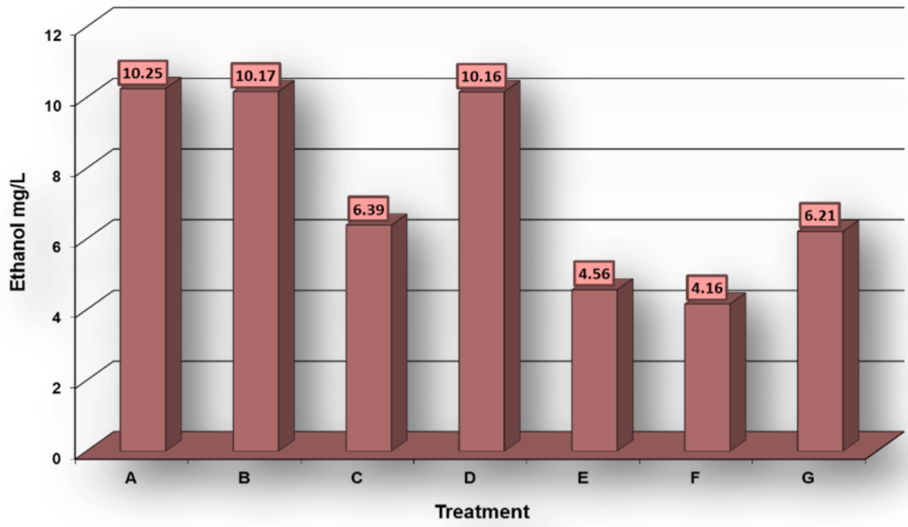

Figure 3. Ethanol content (mg L^{-1}) in yogurt samples experimental treated with threonine and glycine during refrigerated storage (7 days / 4°C) * (P≤0.05)

Sensory Properties

The results in (table 3) showed the treatments (E) threonine 3g/ L, (F) threonine 3g + glycine 1g/L were the highest in flavor, texture, appearance and color values compared to the rest treatments, because of the excellent flavor caused by the increase in the production of acetaldehyde and the low ethanol and acetone. Retain water and the lack of whey exudation, the consistency of texture and the stability of acidity, which when it rises will cause a great exudation of the whey and thus the collapse of the texture, it preserved the sensory characteristics such as texture, appearance and color without any changes that lead to consumer alienation from the product, and these results match the results obtained (Noviatri *et al.*, 2020; Rul, 2017).

Table3. Sensory properties results of yogurt treated with threonine and glycine during refrigerated storage (28 day / 4°C)

Treatments	1 DAY	7 DAY	14 DAY	21 DAY	28 DAY	LSD value
Flavor (45)						
A	43	42	40	35	32	**5.93 ***
B	42	40	37	34	33	**6.87 ***
C	43	43	40	39	38	**5.21 NS**
D	42	41	38	35	33	**6.07 ***
E	44	44	43	42	41	**4.68 NS**
F	43	42	42	41	40	**4.07 NS**
G	40	40	37	34	32	**6.21 ***
LSD value	**4.58 NS**	**4.09 NS**	**5.07 ***	**5.85 ***	**6.31 ***	**- --**
Body and Texture (30)						
A	30	27	27	23	21	**5.86 ***
B	30	28	25	24	23	**5.72 ***
C	29	28	27	24	24	**4.98 ***
D	27	27	25	23	22	**4.83 ***
E	29	28	26	25	24	**4.78 ***
F	28	28	27	26	25	**3.81 NS**
G	30	27	23	22	21	**6.18 ***
LSD value	**4.28 NS**	**3.61 NS**	**4.69 ***	**0.403 NS**	**0.462 NS**	**---**
Appearance(15)						
A	14	13	12	11	9	**3.97 ***
B	13	12	11	10	9	**4.02 ***
C	14	13	12	12	10	**3.87 ***
D	13	12	11	10	9	**3.61 ***
E	15	13	12	12	10	**4.27 ***
F	15	13	13	12	10	**4.62 ***
G	13	12	11	11	9	**4.08 ***
LSD value	**2.48 NS**	**2.07 NS**	**2.61 NS**	**2.55 NS**	**2.01 NS**	**---**
Color 10						
A	10	10	9	9	9	**1.26 NS**
B	10	10	9	9	9	**1.07 NS**
C	10	10	9	9	8	**2.07 NS**
D	10	10	9	9	9	**1.37 NS**
E	10	10	9	9	9	**1.37 NS**
F	10	10	9	9	9	**1.26 NS**
G	10	10	9	9	9	**1.42 NS**
LSD value	**1.53 NS**	**1.27 NS**	**1.19 NS**	**1.33 NS**	**1.26 NS**	**---**
*** (P≤0.05), NS: Non-Significant.**						

***(A)Control, (B)Glycine 1 g/L, (C)Threonine 1g/ L, (D)Glycine 3g/L, (E)Threonine 3g/ L,(F) Threonine 3g+Glycine 1g/L,(G)Glycine 3g+threonine 1g/L.**

Conclusions

Possibility of using threonine and glycine in the production of yogurt. Improving flavor compounds in yogurt (increasing acetaldehyde content and decreasing acetone and ethanol content), increasing viability of yogurt starter under study, improving the sensory properties flavor, texture and appearance of the yogurt treated with threonine and glycine.

Conflict of Interest

There are no conflicts of interest regarding the publication of this manuscript.

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