

Studies on the production of synbiotic yoghurt

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Abstract: The manufacture possibility of bioyoghurt fortified with different levels of a physiological active ingredient, namely γ -aminobutyric acid (GABA), was aimed to study in relation to bacterial, biochemical, physical, rheological and organoleptical attributes of yoghurt made using milk inoculated with probiotic bacterial starter culture (BSC) *versus* ordinary yoghurt inoculated with yoghurt bacterial starter culture as a control. Milk was firstly adjusted to 3 % fat and fortified with GABA preparation at the level of nil, 100, 150 or 200 mg/kg. Then fortified milks were heat treated to 85°C for 5 min. followed by temperature adjustment to 42°C. Milk preparations were converted into yoghurt, where they were inoculated with 2% of freshly activated YC-X11 or ABT-2, filled into 100 ml polystyrene containers, covered, and incubated at the same temperature degree (42°C) until complete coagulation (through about 3 h.). Thereafter, the containers were transferred to the refrigerator (5±1°C), where they were kept for periodically analyses along 3 weeks. The obtained results declared that, the proportional fortification of yoghurt milk with GABA was associated with significantly gradual increment in the counts of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lb. acidophilus*, *Bifidobacterium* sp., the levels of titratable acidity (TA) as well as acetaldehyde (AC) and decrement in the values of the pH and diacetyl (DA). However, the organoleptic scores of bioyoghurt were not influenced by the fortification level with GABA. The penetration value increased and consistency coefficient (CC), yield stress (YS) decreased as GABA was added. Moreover, the ordinary yoghurt was distinguished with higher count of *Str. thermophilus*, TA%, AC, DA, CC as well as YS; and lower values of pH. Along the cold storage period (CSP) of yoghurt, all BSC strains reached their higher count at the 1st week then reduced in the 2nd week. Similar trends were behaved by DA content of yoghurt. Gradual increase in TA%, CC, YS, dynamic viscosity and decrease in pH, AC and penetration values were occurred as the CSP of yoghurt was prolonged. Nevertheless, all yoghurt samples, without any exception, were organoleptically accepted until the end of the CSP (3 weeks). Finally, the foregoing results led satisfactory to conclude that, yoghurt beyond its ability to be probiotic food *via* culturing with the gut strains, it could further carry healthier benefits when it was fortified with such physiological active ingredient, namely γ -aminobutyric acid at any level studied.

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1. Introduction

The dairy industry offers a wide range of products from raw milk to the specialist products aimed at special market or consumers and offers ingredients to other industries. Ever-growing consumer demand for convenience, combined with a healthy diet and preference for natural ingredients has led to a growth in functional beverage markets. Current trends and changing consumer needs indicate a great opportunity for innovations and developments in fermented milks. Scientific and clinical evidence is also mounting to corroborate the consumer perception of health from fermented milks. Yoghurt is one of the most popular fermented milk products worldwide and has gained widespread consumer acceptance as a healthy food (Mckinley, 2005).

Yoghurt is coagulated milk product obtained by lactic acid fermentation through the action of *Lactobacillus delbrueckii* ssp. *bulgaricus* and

Streptococcus thermophilus, form milk and milk products (pasteurized or concentrated milk) with or without optional additions (milk powder, skim milk powder, whey powder etc.). The microorganisms in the final products must be viable and abundant (FAO/WHO, 1977).

In recent years some yoghurt products have been reformulated to include live cells of strains of *Lb. acidophilus* and species of *Bifidobacterium* (known as ABT-cultures) in addition to the conventional yoghurt organisms, *Str. thermophilus* and *Lb. delbrueckii* ssp. *bulgaricus*. Therefore, bio-yoghurt is yoghurt that contains live gut microorganisms, the presence of which may give rise to claimed beneficial health effects (Lourens-Hattingh and Viljoen, 2001; Hussein *et al.*, 2008 and Fayed *et al.*, 2011a and b). In order to exert its probiotic effect, the number of viable cells of probiotic bacteria in bio-yoghurt should

exceed 1 million (10^6) (Speck, (1978), (10^8 - 10^9 cfu/g) (Rybka and Kailasapathy, 1995).

Probiotics, prebiotics, synbiotics and associated ingredients also add an attractive dimension to cultured dairy products. A prebiotic is a nondigestible component which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of colonic bacteria, thereby improving the health of the host. Examples- insulin, garlic, onions, chicory root, Asparagus, whole wheat, rye, barley. Foods containing the combination of probiotics and prebiotics are referred to as synbiotics (Fayed *et al.*, 2012a and b).

On the other hand, γ -Aminobutyric acid (GABA) is an amino acid that has long been reported to lower blood pressure by intravenous administration in experimental animals (Takahashi *et al.*, 1955; Stanton, 1963; Lacerda *et al.*, 2003 and Fayed, 2015) and in human subjects (Elliott and Hobbiger, 1959). GABA is present in many vegetables and fruits but not in dairy products. However, the effect of dietary GABA has attracted little attention as a factor that may influence blood pressure. A novel fermented milk product containing GABA was reported to lower blood pressure in people with mild hypertension (Inoue *et al.*, 2003). Hayakawa *et al.*, (2004) investigated the blood pressure-lowering effects of GABA and a GABA enriched fermented milk product (FMG) by low-dose oral administration to spontaneously hypertensive and normotensive Wistar-Kyoto rats and it was suggested that low-dose oral GABA has a hypotensive effect in spontaneously hypertensive and finally concluded that the hypotensive effect of FMG was due to GABA.

Therefore, the objective of the present research was to study manufacture possibility of yoghurt with different levels of GABA in relation to bacterial, biochemical, physical, rheological and organoleptic attributes of yoghurt made using milk inoculated probiotic bacterial starter culture (BSC) versus ordinary BSC as a control.

2. Materials and Methods

2.1. Materials

Fresh cow s milk was obtained from Dairy Science Department, Faculty of Agriculture, Cairo University, Egypt. The chemical composition determined in the milk used in this study was 12.0 % total solids, 3.5 % fat and 3.3 % protein. The titratable acidity was 0.16 % as lactic acid and the pH value was 6.7.

Freeze dried conventional yoghurt starter culture (FD-DVS YC-X11-Yo-Flex) containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* as well as freeze dried probiotic starter culture (FD-DVS ABT-2-Probio-Tec) containing *Lb.*

acidophilus, *Bifidobacterium* sp. and *Str. thermophiles* were obtained from Chr. Hansen A/S, DK-2970 Horsholm, Denmark.

Γ -Aminobutyric acid (GABA) preparation (97%), EEC No:200-258-6, LOT No:10154342, produced by Alfa Aesar GmbH& KG, Karlsruhe, Germany was obtained from the local market.

2.2. Experimental procedures

Preparation of bacterial starter culture

Lyophilized bacterial cultures were separately inoculated in previously autoclaved ($121^\circ\text{C}/15\text{min.}$) skimmed milk and incubated at 42°C for the YC-X11 or at 37°C for the ABT-2 type. The complete curdling occurred within 8h., the bacterial starter cultures were freshly used.

Preparation of fermented milk

Milk was firstly adjusted to 3% fat and fortified with γ -aminobutyric acid preparation at the level of nil, 100, 150 or 200 mg/kg (as recommended by Hayakawa *et al.*, (2004). Then fortified milks were heat treated to 85°C for 5 min. followed by temperature adjustment to 42°C . Milk preparations were converted into yoghurt according to the protocol proposed by Tamime and Robinson (1999) with adopting the manufacture conditions enacted by EOSQ (2010), where they were inoculated with 2% of freshly activated YC-X11 or ABT-2 bacterial starter culture. The inoculated milk samples were aseptically transferred into 100 ml polystyrene containers tightly sealed and incubated at the same temperature degree (42°C) until complete coagulation (through about 3 h.). Thereafter, the cups were transferred to the refrigerator ($5\pm 1^\circ\text{C}$), where they were kept for 3 weeks and analyzed at fresh and after 1, 2 and 3 weeks of storage, respectively. Three replicates were done from the whole experiment.

2.3. Analytical methods

Dry matter, total nitrogen and ash contents as well as titratable acidity (TA) % were determined according to AOAC (2007). Fat content was determined as in Ling (1963). Acetaldehyde and diacetyl contents ($\mu\text{mol/g}$) were determined according to Lees and Jago (1969) and (1970) respectively. The pH value was measured electrometrically using Lab. pH meter with a glass electrode, Hanna model 8417 digital pH meter.

Rheological properties of fermented milk were measured at 10°C using a rotary viscometer (RHEOTEST, type RV and Pruefgeraetewerk Medingn, Dresden) as described by Toledo (1980) consistency coefficient were calculated from the descending flow curve to express the data corresponding the stirred product using the following equation:

$$\text{Log } \delta = \text{log } \kappa + n \text{ log } \gamma$$

Where:

δ = Shearing stress, γ = shearing gradient or shear rate, n = Flow behavior index and κ = consistency coefficient or consistency index

Whiles, the yield value or yield stress and apparent viscosity were calculated by fitting the shear stress-shear rate data of the descending flow curve to the Casson equation (**Bourne, 1982**):

$$\sqrt{\delta} = \sqrt{\delta_0} + \eta a \sqrt{\gamma}$$

Where:

δ = Shearing stress, δ_0 = yield stress,

ηa = apparent viscosity and γ = shear rate

Dynamic viscosity was calculated at shear rate of 10.48 sec⁻¹.

The count of *Lactobacillus delbrueckii* ssp. *bulgaricus* was enumerated using MRS agar medium as described by **Gucimonde et al., (2003)**. Culture was incubated anaerobically for 2 days at 37°C. Whilst, *Streptococcus thermophilus*, *Lb. acidophilus* and *Bifidobacterium* sp. were enumerated using M17 agar, MRS-salicin agar and MRS agar media supplemented with 0.05% L-cystein and 0.3% Lithium chloride, respectively after the incubation at 37°C for 72 h. as in **Dave and Shah (1996)**. The count was expressed as colony forming units (cfu/g) of product.

Sensory evaluation of yoghurt samples was applied for storage period by regular score panels including the staff members of Dairy Research Department, Food Technology Research Institute, agricultural Research Center according to **Tamime and Robinson (1999)** using the yoghurt evaluation scheme III approved by the American Dairy Science Association.

The data obtained were exposed to proper statistical analysis according to statistical analysis system user's guide (**SAS, 1996**).

3. Results and Discussion

3.1. Gross composition

Data present in Table (1) revealed that, most determined yoghurt compositional criteria, namely dry matter (DM), fat, protein and lactose contents did not exhibit any significant differences among all treatments, while the ash content trended to reduce significantly as the GABA level increased. It is worthy to mention that, all obtained DM and fat contents in this study are, in general, in coincidence with the **Egyptian Organization for Standards and Quality (EOSQ, 2010)**. Similar observations were reported by **Fayed et al., (2011a and b and 2012a)**.

Table (1): Gross composition of bioyoghurt (ABT-2) as affected by the fortification level with gamma amino butyric acid (GABA) versus the ordinary yoghurt (YC-XII) as a control.

Property	YC-XII		ABT-2		
	Control	Nil	GABA		
%	Control	Nil	100mg/kg	150mg/kg	200mg/kg
Dry matter	15.87	15.86	15.89	15.91	15.92
Fat	3.5	3.5	3.4	3.5	3.4
Protein (T.Nx6.38)	5.38	5.36	5.37	5.37	5.37
Lactose*	5.041	5.141	5.231	5.086	5.373
Ash	1.029 ^{ab}	1.019 ^a	0.999 ^{bc}	1.004 ^{bc}	0.987 ^c

*Calculated by the difference.

The means with the same letter did not significantly differ (P>0.05).

3.2. Population of some strains of lactic acid bacteria

Values illustrated in (Table 2) indicated that, the fortification of yoghurt milk with 100 followed by 150 mg/kg GABA led significantly to promote the growth of *Streptococcus thermophiles* and *Lactobacillus acidophilus* whether when fresh or along 3 weeks of cold storage period. While the latter level (150mg/kg) encouraged the growth of *Bifidobacterium* sp. along the cold storage period of yoghurt followed by 100 or 200 mg GABA/kg. These results are more or less in complete agreement with findings reported by **Fayed et al (2012b)** and **Fayed (2015)**, who confirmed that, the growth of *Str. thermophilus*, *Lb. delbrueckii* ssp. *bulgaricus* or *Lb. acidophilus* in yoghurt was supported by GABA. While, the growth of

Bifidobacterium sp. was also stimulated by GABA but a lower extent. They added also that, the counts of all strains, without any exception, were significantly increased as the fortification level was raised. Further, it is worthy to mention that, each bacterial count of all yoghurt treatments, in relation to the GABA fortification level, whether when fresh or along cold storage period, stilled conforming the figures provided by **Codex Alimentarius Commission of FAO/WHO (2002)** which approved an international standard as established a minimum of 10⁷cells/g for the starter cultures of fermented milks and a minimum of 10⁶cells/g for specific starter bacteria for which a claim is made for specific microorganism that has been added as supplement.

3.3. Titratable acidity% and pH value

The acidity contents as well as pH values (Table 3) did not significantly influenced by the kind of the bacterial starter used but the acid production was increasingly promoted when the GABA level in bioyoghurt was 150 mg/kg. Moreover, gradual increment in the acidity content and hence decrement in the pH value were associated with the cold storage

period prolonging (Table 3). Fayed *et al.*, (2012a) found that, the GABA containing yoghurt exhibited higher TA% and hence lower pH value *versus* those of the control. These results trends are in harmony with those of LAB count mentioned before in Table (2). The increasing of the fortification level with GABA was associated with TA% increment and consequently low pH value.

Table (2): Population of some strains of lactic acid bacteria (log count cfu/g) of bioyoghurt (ABT-2) as affected by the fortification level with gamma amino butyric acid (GABA) during cold storage period *versus* the ordinary yoghurt (YC-XII) as a control.

Bacterial strain	Type of yoghurt				
	YC-XII Control	ABT -2			
		GABA fortification level (mg/kg)			
		Nil	100	150	200
	Fresh				
<i>Streptococcus thermophilus</i>	7.70±0.02 ^b	7.51±0.02 ^c	7.81±0.02 ^a	7.75±0.02 ^{ab}	7.71±0.10 ^b
<i>Lactobacillus bulgaricus</i>	7.74±0.02	ND	ND	ND	ND
<i>Lactobacillus acidophilus</i>	ND	7.73±0.02 ^b	7.81±0.02 ^a	7.71±0.09 ^b	7.73±0.03 ^b
<i>Bifidobacterium sp.</i>	ND	7.83±0.04 ^b	7.86±0.05 ^{ab}	7.91±0.02 ^a	7.88±0.02 ^{ab}
	1 week				
<i>Streptococcus thermophilus</i>	7.89±0.02 ^{ab}	7.78±0.03 ^c	7.92±0.03 ^a	7.84±0.03 ^b	7.86±0.05 ^{ab}
<i>Lactobacillus bulgaricus</i>	7.95±0.05 ^a	ND	ND	ND	ND
<i>Lactobacillus acidophilus</i>	ND	7.83±0.06 ^{bc}	7.94±0.05 ^a	7.90±0.03 ^{ab}	7.82±0.03 ^c
<i>Bifidobacterium sp.</i>	ND	7.93±0.03 ^a	7.93±0.03 ^a	7.96±0.02 ^a	7.96±0.02 ^a
	2 weeks				
<i>Streptococcus thermophilus</i>	8.05±0.06 ^a	7.81±0.09 ^b	7.94±0.13 ^{ab}	7.89±0.03 ^b	7.93±0.03 ^{ab}
<i>Lactobacillus bulgaricus</i>	9.07±0.05 ^a	ND	ND	ND	ND
<i>Lactobacillus acidophilus</i>	ND	7.92±0.11 ^b	8.03±0.03 ^a	7.94±0.03 ^{ab}	7.94±0.03 ^{ab}
<i>Bifidobacterium sp.</i>	ND	7.86±0.11 ^{ab}	7.80±0.02 ^b	7.91±0.04 ^a	7.85±0.04 ^{ab}
	3 weeks				
<i>Streptococcus thermophilus</i>	7.78 ± 0.02 ^a	7.76±0.05 ^a	7.81±0.04 ^a	7.82±0.03 ^a	7.82±0.06 ^a
<i>Lactobacillus bulgaricus</i>	7.90±0.02 ^a	ND	ND	ND	ND
<i>Lactobacillus acidophilus</i>	ND	7.83±0.03 ^{ab}	7.84±0.04 ^{ab}	7.79±0.10 ^b	7.83±0.03 ^{ab}
<i>Bifidobacterium sp.</i>	ND	7.68±0.10 ^b	7.75±0.02 ^{ab}	7.79±0.02 ^a	7.74±0.04 ^{ab}

ND: Not determined

The means with the same did not significantly differ (P>0.05).

3.4. Carbonyl components:

Concerning the level of carbonyl components, bioyoghurt containing 200 mg GABA/kg was distinguished with a high acetaldehyde (AC) content after that of the ordinary yoghurt, while the control bioyoghurt came in the last order (Table 4).

Likewise, the ordinary yoghurt possessed the highest diacetyl (DA) level followed by any one of bioyoghurt regardless the GABA level. *i.e.* the fortification with GABA by any level led to delay the diacetyl formation in the yoghurt.

As the cold storage of yoghurt was progressed the acetaldehyde content of all yoghurt treatments gradually decreased. While the diacetyl reached its highest level at the 1st week of cold storage of all treatment without any expectation and then trended to reduce by prolonging the cold storage period. Rasic and Kurmann (1978) reported

that, a decrease in the AC content occurs during the storage of yoghurt from the beginning 5 h. after fermentation is complete. Whereas, AC could be presumably reduced to ethanol and/or transferred to DA. Similar findings were reported by El-Kenany (1996), Mistry (2002), Husein *et al.*, (2006), Fayed *et al.*, (2012a and b) and Aita *et al.*, (2015).

Table (3): Titratable acidity% and pH value of bioyoghurt (ABT-2) as affected by the fortification level with gamma amino butyric acid (GABA) during cold storage period versus the ordinary yoghurt (YC-XII) as a control.

Cold storage period (Week)	YC-XII	ABT-2			
	Control	Nil	100mg/kg	150mg/kg	200mg/kg
GABA					
Titratable acidity % (as lactic acid)					
Fresh	0.92 ^{ab,d}	0.84 ^{a,d}	0.89 ^{b,d}	0.95 ^{ab,d}	0.79 ^{ab,c}
1	1.09 ^{a,c}	0.92 ^{a,c}	0.96 ^{b,c}	1.05 ^{ab,c}	0.90 ^{ab,bc}
2	1.15 ^{ab,b}	1.10 ^{a,b}	1.02 ^{c,b}	1.09 ^{c,b}	0.95 ^{bc,b}
3	1.29 ^{ab,a}	1.21 ^{a,a}	1.15 ^{c,a}	1.20 ^{bc,a}	1.13 ^{abc,a}
pH value					
Fresh	4.73 ^{a,a}	4.74 ^{a,a}	4.67 ^{a,a}	4.63 ^{a,a}	4.62 ^{a,a}
1	4.61 ^{a,b}	4.60 ^{a,b}	4.54 ^{a,b}	4.50 ^{a,b}	4.48 ^{a,b}
2	4.49 ^{b,c}	4.51 ^{b,c}	4.50 ^{a,b}	4.46 ^{ab,bc}	4.44 ^{ab,b}
3	4.40 ^{a,d}	4.45 ^{a,c}	4.43 ^{a,c}	4.42 ^{a,c}	4.40 ^{a,b}

The letters before comma possess the factor of GABA level. While those after comma possess the factor of the cold storage period. The means with the same letter at any position did not significantly differ ($P>0.05$).

Table (4): Acetaldehyde and Diacetyl ($\mu\text{mol/g}$) of bioyoghurt (ABT-2) as affected by the fortification level with gamma amino butyric acid (GABA) during cold storage period versus the ordinary yoghurt (YC-XII) as a control.

Cold storage period (week)	YC-XII	ABT-2			
	Control	Nil	100mg/kg	150mg/kg	200mg/kg
GABA					
Acetaldehyde ($\mu\text{mol/g}$)					
Fresh	333.6 ^{ab,a}	271.3 ^{b,a}	279.9 ^{ab,a}	285.8 ^{a,a}	292.4 ^{ab,a}
1	250.5 ^{b,b}	255.1 ^{ab,b}	264.4 ^{a,b}	279.7 ^{ab,b}	280.9 ^{ab,b}
2	235.2 ^{bc,c}	240.9 ^{c,c}	250.7 ^{ab,c}	264.4 ^{a,c}	270.2 ^{a,c}
3	210.8 ^{b,d}	230.2 ^{b,d}	239.8 ^{a,d}	250.6 ^{a,d}	258.9 ^{a,d}
Diacetyl ($\mu\text{mol/g}$)					
Fresh	15.51 ^{a,d}	13.52 ^{ab,d}	13.55 ^{b,d}	13.57 ^{b,d}	13.59 ^{b,d}
1	22.62 ^{a,a}	15.69 ^{a,a}	15.72 ^{a,a}	15.75 ^{a,a}	15.78 ^{a,a}
2	20.73 ^{a,b}	14.84 ^{a,b}	14.92 ^{a,b}	14.96 ^{a,b}	14.98 ^{a,b}
3	17.08 ^{a,c}	14.40 ^{a,c}	14.44 ^{a,c}	14.48 ^{a,c}	14.50 ^{a,c}

The letters before comma possess the factor of GABA level. While those after comma possess the factor of the cold storage period. The means with the same letter at any position did not significantly differ ($P>0.05$).

3.5. Rheological properties:

As could be seen in Table (5), the ordinary yoghurt exhibited the strongest resistant to penetrate *versus* the bioyoghurt. Whereas the penetration value of 100mg GABA/kg was the highest. At the end of cold storage period (3 weeks), the resistance to penetration at all treatment was restored, the lowest penetration values were found in those bioyoghurt of 100 or 200 mg GABA/kg, even that of the ordinary yoghurt.

Concerning the consistency coefficient (CC) and yield stress (YS), data revealed that, significantly gradual reductions were occurred in both criteria as the portion of GABA increased. Moreover, the yoghurt cultured with (YC-XII) starter culture exhibited CC, YS and dynamic viscosity values higher than gained for the bioyoghurt. This phenomenon was observed in the products whether when fresh or during the CSP. That could be positively depending to the difference in TA%. Similar observations were reported by Hussein *et al.*, (2008) and Fayed *et al.*, (2011a,b, 2012a and b).

Along the CSP, both of CC, YS and dynamic viscosity values restored as the CSP of yoghurt prolonged. Those findings agree with those previously reported by Rhom and Schmid (1993), Fayed *et al.*, (1996), Tamime and Robinson (1999), Husein *et al.*, (2006), Fayed *et al.*, (2011 a, b, 2012a and b) and Aita *et al.*, (2015).

Table (5): Rheological properties of bioyoghurt (ABT-2) as affected by the fortification level with gamma amino butyric acid (GABA) during cold storage period versus the ordinary yoghurt (YC-XII) as a control.

Cold storage period (week)	YC-XII	ABT-2			
	Control	Nil	100mg/kg	150mg/kg	200mg/kg
Penetration value (mm)					
Fresh	24.96 ^{c,a}	25.62 ^{ab,a}	25.60 ^{a,a}	25.59 ^{ab,a}	25.56 ^{b,a}
3	23.36 ^{b,b}	23.73 ^{a,b}	22.79 ^{c,b}	22.70 ^{d,b}	21.95 ^{e,b}
Consistency coefficient (dyne-sec/cm ²)					
Fresh	416.45 ^{a,b}	324.79 ^{e,b}	326.51 ^{d,b}	328.55 ^{c,b}	330.59 ^{b,b}
3	589.92 ^{a,a}	341.02 ^{b,a}	344.81 ^{b,a}	348.62 ^{b,a}	350.79 ^{b,a}
Yield stress (dyne /cm ²)					
Fresh	177.84 ^{a,b}	129.82 ^{b,b}	120.50 ^{c,b}	110.45 ^{d,b}	105.92 ^{e,b}
3	190.51 ^{a,a}	144.73 ^{e,a}	170.89 ^{b,a}	160.93 ^{c,a}	151.36 ^{d,a}
Dynamic viscosity (centipoise at shear rate 10.48 sec ⁻¹)					
Fresh	43.66 ^{a,b}	16.95 ^{b,b}	16.98 ^{b,b}	16.99 ^{b,b}	17.02 ^{b,b}
3	48.71 ^{a,a}	19.91 ^{c,a}	19.94 ^{c,a}	20.01 ^{c,a}	21.01 ^{b,a}

The letters before comma possess the factor of GABA level. While those after comma possess the factor of the cold storage period. The means with the same letter at any position did not significantly differ ($P>0.05$).

3.6. The organoleptic quality:

As would be observed from the panelists scores given in Table (6), the appearance as well as the body and texture properties of bioyoghurt were not influenced either by the level of milk fortification with GABA or the CSP.

Likewise, the flavor scores did not significantly differed towards all factors studied. Where, the palatability of GABA bioyoghurt was as good as that of the control. Along CSP, the flavor score was significantly the same unchanged.

According to the forgoing score of previous sensory criteria of samples among all factors studied, the directions of overall scores of all treatments were as high as the ordinary yoghurt (the control), whether when fresh or along the CSP.

Table (6): Organoleptic score of bioyoghurt (ABT-2) as affected by the fortification level with gamma amino butyric acid (GABA) during cold storage period versus the ordinary yoghurt (YC-XII) as a control.

Cold storage period (week)	YC-XII	ABT-2			
	Control	Nil	100mg/kg	150mg/kg	200mg/kg
Appearance score (out of 5 points)					
Fresh	5	5	5	5	5
1	5	5	5	4	5
2	5	5	5	5	5
3	5	5	5	5	5
Body and texture score (out of 5 points)					
Fresh	5	5	5	5	5
1	5	5	5	5	4
2	5	5	5	5	5
3	5	5	5	5	5
Flavor score (out of 10 points)					
Fresh	10	10	10	10	10
1	10	10	10	10	10
2	10	10	10	10	10
3	10	10	10	10	10
Total score (out of 20 points)					
Fresh	20	20	20	20	20
1	20	20	20	19	19
2	20	20	20	20	20
3	20	20	20	20	20

4. Conclusion

Finally, the foregoing results led satisfactory to conclude that, yoghurt beyond their ability to be probiotic food *via* its culturing with the gut strains, it could further carry more healthy benefits when it was fortified with GABA that act as prebiotic converting the product to be symbiotic.

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