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UTILIZATION OF BREWERS SPENT GRAIN (BSG) IN MAKING FUNCTIONAL YOGHURT

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ABSTRACT

In the present study, the brewers spent grain BSG was used to supplement Yoghurt by displacement cow's milk in ratios 0, 2, 4, 6, 8 and 10%. The proximate chemical composition, polyphenols and total flavonoids in BSG were determined. The resultant yoghurt samples were evaluated chemically, microbiologically and sensory fresh and stored at 4˚C for 3, 7, 10 and 14 days. Significant differences in chemical, microbiological and sensory evaluation have been shown by increasing of BSG level in yoghurt. The addition of 10% BSG had the highest value of Total solids, Protein, Fat, Fiber, Ash and pH values but it had the lowest in Titratable acidity ,microbical count and sensory properties .

INTRODUCTION

Brewers' spent grain (BSG) is obtained from barley) *HordeumBrew vulgare* L.) as the outer pericarp-seed coat layers from the original malted barley grain which remain after hot water extraction at 65–70˚ C (mashing) (Mussatto *et al* .٢٠٠٦),.It can represent approximately 30% (w/w) of the starting malted grain) Townsley ,(١٩٧٩ ,which makes BSG a readily available, high volume and low cost by-product within the brewing industry(Celus *et al* ,. ;٢٠٠٦Forssell *et al* ;٢٠٠٨ ,.Treimo *et al* .٢٠٠٩ ,).Variation in BSG composition can be expected to arise from differences in barley malting cultivars, malting practices, adjuncts added and wort production during mashing processes in breweries(Palmer, 2006; James *et al* .٢٠١٠),.Although Brewer's spent grain (BSG) is the major by-product of the brewing industry and is available in large quantities, its main application has been limited to animal feeding. Recently, attempts have been made to use BSG as a source of phenolic acids (Bartolome´*et al* ;٢٠٠٢ ,.Mussatto *et al* ٢٠٠٦ ,.and Mallouchos *et al* ٢٠٠٧),. .There is approximately 3.4 million tons of BSG produced annually in the European Union according to Eurostat Dat (Mussatto *et al* ٢٠٠٦ ,and McCarthy *et al* .٢٠١٢),BSG is of low cost and high nutritive value. The ingestion of BSG, or its derived products, has health benefits. Incorporation of BSG into rat diets is beneficial to intestinal digestion, alleviating both constipation and diarrhoea .Such effects were attributed to the content of glutamine-rich protein, and to the high content of non-cellulosic polysaccharides and smaller amounts of B-glucan) Mussatto ;٢٠٠٦ ,Tang *et al* .(٢٠٠٩ ,.BSG may provide a number of benefits when incorporated into human diets such as for the prevention of certain diseases including cancer , gastrointestinal disorders, diabetics, and coronary heart disease) Aman *et al* ,. ١٩٩٤and Jacobs *et al* .١٩٩٨).

Yoghurt is a dairy product produced by bacterial fermentation of milk. Generally, the bacteria used to make Yoghurt are cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yoghurt is a fermented dairy product; beyond its nutritional characteristics and importance for human diet it is not being considered as a significant source of phenolic compounds (O'Connel and Fox, 2001 and Karaaslan *et al.,* 2011)). Also, lactic acid bacteria and its metabolites have shown to play an important role in improving microbiological quality and shelf-life of many fermented food products. Traditional preparation ofYogurt may be beneficial by including other ingredients such as soya protein, vegetables, sweet potato, pumpkin and plum to enhance the flavour as well as the nutritional quality(Joo *et al.,* 2001; Park *et al.,* 2003).

Additives had been used to enhance the phenolic content of yoghurt (Aportela *et al*., 2005; Vasiljevic *et al*., 2007, Peng *et al*., 2009 and Sendra *et al*., 2010).

Yoghurt is considered as a functional food because of its lactic acid bacteria (LAB) that provide significant therapeutic values during milk fermentation including the highly digestible nutrients (Deeth and Tamime, 1981; Marshal *et al*., 1986; Gu¨ ler-Akın and Akın, 2007) as well as the ability to produce various antimicrobial compounds (Temmerman *et al*., 2002) reduce serum cholesterol (Jackson *et al*., 2002), alleviate lactose intolerance (De-vrese *et al*., 2001) and stabilize gut microflora (Gibson *et al*., 1997).

Many addition have been added to Yoghurt to increase its nutrition value e.g. oat, rice, soy and maize flour (Hess, 1997), wheat and bamboo fiber (Dello *et al*., 2004), orange fiber (Garcia *et al*., 2006; Sendra *et al*., 2010), green and red tea (Imene-Jaziri *et al*., 2009), pachrhizus erosus fiber (Ramirez-Santiago *et al*., 2010), dibs (El-Nagga and Abd El–Tawab, 2012), fish collagen (Shori *et al*., 2013)and black waxy rice bran (Nontasan *et al*., 2012).

The main objective of this study was to evaluate the brewers spent grain as industrial waste of brewers' plant and fortify Yoghurt by bioactive natural compounds that present in brewers spent grain and evaluated the chemical, microbiological and sensory evaluation of yoghurt during storage periods.

MATERIALS AND METHODS

Spent grain was obtained from Al-Ahram Company, EL-Sharqeia Governorate ,Egypt .Fresh Cow's milk was obtained from the herds of Nasser Agricultural Secondary School ,Damanhour ,Behera Governorate, Egypt . Yoghurt culture Direct vat Set (DVS) of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* sp .*bulgaricus* in the ratio (1:1) were obtained from Chr. Hansen's Lab., Copenhagen, Denmark. The cultures were propagated in sterilized skim milk and incubated at 37ºC for 24 hrs. Sucrose, Strawberry as flavoring agent and Plastics cups (100 – 120 gm (which used in this study (made from polystyrene and polyester) was obtained from local market , Damanhour ,Behera Governorate, Egypt .

HCl, ammonium nitrate, potassium chloride, sodium chloride, sodium carbonate, sodium acetate, Foline Ciocalteu reagent, sodium nitrate, ammonium iron sulphate and aluminum chloride were purchased from Merck Chemical Company, Germany and reagent-grade Gallic acid, were purchased from Sigma Chemical Co.

Spent grain was dried at 50˚ C / 24 h then milled in laboratory mill)National, Japan) and passed through ٤٠ mesh sieve and kept frozen in glass jars at –18˚C until used Charilaos *et al* .(٢٠٠٩) ,.Moisture, protein, fat and ash content were determined in Brewer's spent grain (BSG ,(by official methods were performed AOAC, (2007 .(The samples were investigated for their in vitro protein digestibility by the modified method of Hsu *et al* .(١٩٧٧) ,.

The total dietary fiber content was determined using a combination of enzymatic and gravimetric methods (Sigma-Aldrich Inc., St. Louis, MO, USA) AOAC, (2007). Milled and dried samples were gelatinized with heat stable α-amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch present in the sample. Ethanol was added to precipitate the soluble dietary fiber. The residue was then filtered and washed with ethanol and acetone. After drying, the residue was weighed. Half of the samples were analysed for protein and the others ash. Total dietary fiber is the weight of the residue less the weight of the protein and ash.

The total phenolic content was determined according to the Folin-Ciocalteu procedure (Zilic *et al.*, 2012). Briefly, the extract (100 µl) was transferred into a test tube and the volume adjusted to 500 µl with distilled water and oxidized with the addition of 250 µl of Folin-Ciocalteau reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous Na2CO3 solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as mg of Gallic acid equivalent (GAE) per100 g of sample.

The total flavonoid content was determined according to (Zilic *et al.* 2012). Briefly, 50 µl of 5% NaNO2 was mixed with 100 µl of extract. After 6 min, 500 µl of a 10% AlCl3 solution was added. After 7 min, 250 µl of 1 M NaOH was added, and the mixture was centrifuged at 5000 *g* for 10 min. Absorbance of the supernatant was measured at 510 nm against the solvent blank. The total flavonoid content was expressed as mg of catechin equivalent (CE) per g of sample.

The pH values of brewers spent grain (BSG (were determined using a digital pH meter) Mettler Toledo 320) at room temperature (20 ±1˚C). Spent grain was mixed with distilled water (1:1 ml) before pH measurement as outlined by AOAC, (2007.(

Titratable acidity (TA%) was determined by titrating spent grain: distilled water (1:9) mixture using 0.1 N NaOH. TA% was calculated as 1 ml NaOH (0.01 N) neutralizes 0.009 g of lactic acid, AOAC, (2007).

Total bacterial counts were enumerated using plate count agar as described by Messer *et al*.(١٩٨٥) ,.

Detection of Coliform bacteria, Molds and Yeasts were detected as mentioned by APHA, (1992).

For the preparation of Yoghurt, cow's milk (4.5% fat), strawberry and sucrose were added to raw milk, the milk with flavor and sugar were heated up to 95 ±1 ^{0}C / 10 min and powder of spent grain was added in 5 ratio (2% - 4% - 6% -8% - 10%), then it was struck twice in the mixer for 3 minutes. Milk samples were cooled to 42 ±1^{0}C, and the prepared starter was added (3%). The samples were placed into 100 mL plastic cups. The cups were incubated at 42 ±1^{o}C until complete coagulation (3h). After fermentation, yoghurt samples were cooled to 4 ±1^{o}C and stored at this temperature for 14 days for further analysis. Control yoghurt was prepared following the same methodology without the addition of spent grain powder. Three replicate were done from whole experiment (Hassanein and Somaya-Moursy, 2008).

Moisture content bulletin no.925.09, fat content bulletin no.923.05, Protein content bulletin no.979.09 and Ash content bulletin no. 923.03 were determined in Yoghurt by official methods were performed (Ling, 1963). The pH values and Titratable acidity of Yoghurt were determined according to AOAC, (2007).The total phenolic content of Yoghurt was determined according to Zilic *et al.*, (2012).

For the microbiological examination, one gm of Yoghurt sample was added to 9ml of sterile distilled water, thoroughly mixed using vortex apparatus to make a10^{-1} dilution from which decimal serial dilutions were prepared (APHA, 1992).

Total bacterial counts were enumerated on the standard plate count agar (Messer *et al*., 1985). M17 agar as described by Terzaghi and Sandine (1975) was used to enumerate *Streptococcus thermophilus*, MRS medium was used to enumerate *Lactobacillus delbrueckii* sp. *Bulgaricus* (DeMan *et al*., 1960). Coliform, yeast and molds were detected as described by (APHA, 1992).

All Yoghurt samples were organoleptically evaluated by 12 persons of Staff members of the Dairy Science and Technology Department, Center of Agricultural Research, El-Sabhya, Alexandria, Egypt according to the scheme described by Pearce and Heap, (1974).

All obtained data were statistically analyzed using SAS (2000). Data were analyzed as factorial arrangement of % of spent grain added and storage period in complete randomized design with three replicates. Comparisons among the means of different treatments were achieved using the least significant difference procedure (LSD) at p = 0.05 level as illustrated by Al – Rawi and Khalaf – Allah, (1980).

RESULTS AND DISCUSSION

Chemical composition of Brewers Spent Grain and used cow's milk:

Table (1) shows the chemical composition of dried brewers spent grain and used cow's milk. Data in this respect agree with that published by (Chaudhardy and Weber, 1990 and Sendra *et al*., 2010)

Table (1): **chemical composition of raw materials**

Samples	Moisture %	Crude protein %	Crude fat %	Crude fiber %	Ash %	Carbohydrate*
Dried BSG	٧.٤٠	٢٠.٨٦	٤.٣٤	١٢.١٧	٣.٩٠	٥١.٣٣
Cow's milk	٨٥.٧٥	٣.٨٥	٤.٦	٠	٠.٩٧	٤.٨٣

***by difference**

Data present in Table (2) show the total phenols content of spent grain which figures 1.47%. It was clear that the spent grain contains the highest amount of ferulic acid and sinapic acid content. Also data showed considerable amount of cinnamic acid, syrngic acid, protochatchuic acid, gallic acid and coumarin content of BSG. This result show that the brewers spent grain as industrial waste is a source of natural antioxidant and phenols. Data in this respect agree with that of Chaudhardy and Weber, (1990). Phenolic component of BSG has potential bioactive effects, which are worth pursuing given that the inclusion of BSG into human foodstuffs is viable and beneficial (McCarthy *et al*., 2012).

Table (2): phenols compound in brewers spent grain:

Phenols	BSG
Polyphenols	%١.٤٧
Total phenol (mg GAE/100g)	١٥٢٦.٦٠
Total flavonoids (mg CE/100g)	١٦.٢٠
Gallic acid (mg/100g)	٢٩.٩٦٤
Protochatchuic acid (mg/100g)	٣٩.٩٥٨
Syrngic acid (mg/100g)	٤٩.٢٠٩
Coumarin (mg/100g)	١٧.٧٤٦
Cinnamic acid (mg/100g)	٤٩.٧٠٩
Ferulic acid (mg/100g)	١١٩٦.٠١٥
Sinapic acid (mg/100g)	١٢٧.٧٩٨

As with the chemical composition of Yoghurt present in Table (3) Generally ,the chemical composition of Yoghurt significantly was affected by adding different level of brewers spent grain. Positive correlation was observed between the level of brewers spent grain addition and the chemical composition (total solid, protein, crude fiber, ash, carbohydrate and total phenols). The total solid content increased by 33.98% in the presence of 10% BSG, the protein content increased by 32.62% by adding of 10% BSG, the crude fiber content increased by 100% by adding of 10% BSG, the ash content increased by 24% by adding of 10% BSG, the carbohydrate content increased by 43.16% by adding of 10% BSG and the total phenols content increased by 85.18% by using of 10% BSG .Sendra *et al* (٢٠١٠) ,.found that the total solid content of Yoghurts ranged from 14.35 to 14.85 g/100 g, the fat content was less than 0.1 g/100 g, protein from 4.89 to 5.12 g/100 g and ash from 0.68 to 0.81 g/100 g. Increasing citrus fiber concentration from 0.2 to 1 g/100 g did not significantly affect in total solids, fat, ash and protein contents of yoghurts .El-Nagga and Abd El–Tawab (٢٠١٢) ,postulated that the addition 2% from dibs was not significant they found that the total solid content of yoghurt ranged from 13.34 to 13.60 g/100 g, the fat from 3.10 to 2.90 g/100 g,

protein from 4.48 to 4.11 g/100 g and ash from 0.85 to 1.19 g/100 g . McCarthy *et al* (٢٠١٢) ,reported that BSG may have the potential to be developed as a food additive or dietary supplement with possible health promoting properties.

Table (3): Chemical composition of fresh Yoghurt as affected by adding brewers spent grain

Samples	Total solid(%)	Crude protein(%)	Fat (%)	Crude Fiber(%)	Ash (%)	Carbohydrate*	Total Phenols Mg/100g
C	١٤.٦٥^{f}	٣.٨٠^{d}	٤.٦^{a}	٠.٠٠^{e}	٠.٩٥^{f}	٥.٤٠^{f}	٢٦^{f}
T1	١٦.١٦^{e}	٤.١٥^{cd}	٤.٦^{a}	٠.٢٥^{d}	١.٠١^{e}	٦.١٥^{e}	٥٥.٩٧^{e}
T2	١٧.٦٦^{d}	٤.٥٢^{bc}	٤.٦^{a}	٠.٤٧^{c}	١.٠٧^{d}	٧.٠٠^{d}	٨٥.٩٢^{d}
T3	١٩.١٧^{c}	٤.٩٠^{ab}	٤.٦^{a}	٠.٧٢^{b}	١.١٢^{c}	٧.٨٣^{c}	١١٥.٤٣^{c}
T4	٢٠.٦٨^{b}	٥.٢٧^{a}	٤.٦^{a}	٠.٩٤^{a}	١.١٨^{b}	٨.٦٩^{b}	١٤٥.٦٧^{b}
T5	٢٢.١٩^{a}	٥.٦٤^{a}	٤.٦^{a}	١.٢٠^{a}	١.٢٥^{a}	٩.٥٠^{a}	١٧٥.٣٩^{a}
LSD%	٠.٢٠٥	٠.٣٨٩	N.S	٠.١٤٩	٠.٠١٩	٠.٢٠٩	١٣.٠٤٠

■C -:control
T1:- 2% spent grain + 98% milk **T2:- 4% spent grain + 96% milk**
T3:- 6% spent grain + 94% milk **T4:- 8% spent grain + 92% milk**
T5:- 10% spent grain + 90% milk ***by difference**
Means in a column not sharing the some superscript are significant different at p %٠.٠٥ >
n.s :not significant

Data in Table (4) generally show that there are significant decreases in titratable acidity with increasing the BSG substitution level. Moreover, it was observed that significant and gradual increases were also noticed on increasing the storage periods for all of the treated samples in the present study. The control samples in fresh and during storage were still more than that of rest treated samples in respect to titratable acidity .The changes in Titratable acidity of Yoghurt during storage period could be due to the changes occurred in lactose and protein (soluble nitrogen .(Karaaslan *et al* ,. (٢٠١١)found that the addition of callus extract no significant effect among the experimental and control samples ,El-Nagga and Abd El–Tawab (٢٠١٢) , reported that the addition of 2% dibs ranged between 0.90 to 1.16 and it had a significant effect.

Table (4) :Effect of BSG concentration on the Titratable acidity (%) of fresh and stored Yoghurt samples

Storage period (day)	Treatments						
	C	T1	T2	T3	T4	T5	LSD%
٠	^{a}0.74^{b}	^{ab}0.73^{b}	^{ab}0.73^{c}	^{ab}0.72^{a}	^{bc}0.71^{a}	^{c}0.69^{a}	٠.٠٢٢
٣	^{a}0.75^{c}	^{a}0.74^{ab}	^{a}0.74^{c}	^{b}0.70^{b}	^{bc}0.69^{b}	^{c}0.68^{ab}	٠.٠٢٠
٧	^{a}0.75^{c}	^{a}0.74^{ab}	^{a}0.75^{bc}	^{b}0.70^{b}	^{bc}0.68^{bc}	^{c}0.67^{c}	٠.٠٢٤
١٠	^{a}0.76^{b}	^{a}0.75^{ab}	^{a}0.77^{ab}	^{b}0.69^{b}	^{bc}0.68^{bc}	^{c}0.66^{c}	٠.٠٢٣
١٤	^{a}0.79^{a}	^{b}0.76^{a}	^{ab}0.78^{a}	^{c}0.69^{b}	^{cd}0.67^{c}	^{d}0.64^{d}	٠.٠٢٣
LSD%	٠.٠١٩	٠.٠٢١	٠.٠٢٤	٠.٠٢٢	٠.٠١٨	٠.٠٢٠	

■samples as in Table (3)
Means in a column) between times (not sharing the some superscript are significant different at p %٠.٠٥ >
Means in a raw) between treatments (not sharing the some superscript are significant different at p %٠.٠٥ >

The changes in pH values of Yoghurt samples are shown in Table (5). The pH values are ٤.٧٠ ,٤.٦١ ,٤.٥٥ ,٤.٤٩ ,٤.٤٥ and 4.76 in) control, T1, T2, T3, T4 and T5 ,(respectively at zero time. The trends of all treatments were opposite to that of titratable acidity. The control had the lowest pH values . The pH values increased with increasing the substitution level of spent grain so that T5 was the highest. The pH value of yoghurt slightly decreased gradually during storage period. The decrease in pH values during storage might be due to the changes of Yoghurt component such as lactose and proteins .Amaya-Llanoa *et al* (٢٠٠٨) ,.found that Yoghurt samples with hydrolyzed jicama starches added did not show significant differences , Sendra *et al* (٢٠١٠) ,.postulated that the pH values ranged from 4.58 to 4.71 with the addition 0.2 to 1g/10٠g from citrus fiber ,and it has insignificant effect ,Karaaslan *et al* (٢٠١١) ,.reported that the addition of callus extract was also insignificant difference in the experimental and control samples**.**

Table (5): Effect of BSG adding on pH values of yoghurt samples spent grain during storage periods at 4±1˚C .

Storage period(day)	**Treatments**						
	C	T1	T2	T3	T4	T5	LSD%
٠	^{a}4.45^{a}	^{a}4.49^{a}	^{a}4.55^{a}	^{a}4.61^{a}	^{a}4.70^{a}	^{a}4.76^{a}	N.S
٣	^{a}4.43^{a}	^{a}4.47^{a}	^{a}4.54^{a}	^{a}4.60^{a}	^{a}4.68^{a}	^{a}4.75^{a}	N.S
٧	^{a}4.42^{a}	^{a}4.46^{a}	^{a}4.53^{a}	^{a}4.58^{a}	^{a}4.67^{a}	^{a}4.73^{a}	N.S
١٠	^{f}4.41^{a}	^{e}4.45^{a}	^{d}4.53^{a}	^{c}4.58^{a}	^{b}4.66^{a}	^{a}4.71^{a}	٠.٠٢٠
١٤	^{f}4.40^{a}	^{e}4.43^{a}	^{d}4.51^{a}	^{c}4.56^{a}	^{b}4.64^{a}	^{a}4.70^{a}	٠.٠١٠
LSD%	N.S	N.S	N.S	N.S	N.S	N.S	

■samples as in Table (3 (
Means in a column) between times (not sharing the some superscript are not significant different at p %٠.٠٥ >
Means in a raw) between treatments (not sharing the some superscript are significant different at p %٠.٠٥ >

Brewers Spent Grain was free from microorganism, Coliform bacteria and Molds &Yeasts ,not detected .Our results are in agreement with James *et al*(٢٠١٠) ,.

Results of the microbiological analysis of Yoghurt samples are shown in Table (6 .(Slightly significant effect were generally observed between different treatment concerning Total bacterial count ,counts of *Lactobacillus* and *Streptococcus* during the storage of the resultants Yoghurt up to 14 days at 4 ±1˚C. However, the data showed slight increase in T2 followed by T1, while T5 was of the lowest count .

Data in the same Table also indicated that the Molds & Yeasts and Coliform bacteria were not detected in any treatment either fresh or during storage period. El-Nagga and Abd El–Tawab, (2012) found that the values of *Streptococcus thermophiles* in the presence of 2% from dibs was insignificant but the values of Lactobacilli was significant after 5 and 10 days.

The nondigestible oligo- and polysaccharides, known as dietary fiber, are prebiotics because these compounds are resistant to both digestion and absorption in the human small intestine and may suffer partial fermentation in

the large intestine. The prebiotic also beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving host health (Ainsworth *et al.,* 2007 and Ajanaku *et al.,* 2011).

Table (6): Effect of BSG concentration on the microbial counts (log cfu/ml) of fresh Yoghurt samples and during storage period

Microbial Strain	S.p (D)	Treatments C	T1	T2	T3	T4	T5	LSD%
		Count (cfu /gm)						
Lactobacillus Count (10^7×)	٠	$^{d}7.65^{d}$	$^{c}7.67^{d}$	$^{a}7.70^{d}$	$^{b}7.68^{e}$	$^{e}7.61^{c}$	$^{f}7.58^{c}$	٠.٠١٠
	٣	$^{cd}7.68^{c}$	$^{bc}7.71^{c}$	$^{a}7.80^{b}$	$^{b}7.74^{c}$	$^{d}7.65^{b}$	$^{d}7.65^{b}$	٠.٠٣٤
	٧	$^{c}7.83^{a}$	$^{b}7.87^{a}$	$^{a}7.91^{a}$	$^{b}7.88^{a}$	$^{c}7.81^{a}$	$^{d}7.78^{a}$	٠.٠٢١
	١٠	$^{ab}7.79^{b}$	$^{b}7.77^{b}$	$^{a}7.82^{b}$	$^{a}b7.79^{b}$	$^{c}7.57^{d}$	$^{d}7.52^{d}$	٠.٠٣٥
	١٤	$^{d}7.68^{c}$	$^{c}7.69^{c}$	$^{a}7.74^{c}$	$^{b}7.71^{d}$	$^{e}7.55^{e}$	$^{f}7.49^{e}$	٠.٠٠٨
LSD%		٠.٠٢٤	٠.٠٢٨	٠.٠٢١	٠.٠٢٢	٠.٠٢	٠.٠٢٣	
Streptococcus Count (10^7×)	٠	$^{d}8.40^{c}$	$^{c}8.43^{c}$	$^{a}8.51^{c}$	$^{b}8.49^{c}$	$^{e}8.37^{c}$	$^{f}8.31^{c}$	٠.٠١٠
	٣	$^{d}8.44^{b}$	$^{c}8.47^{b}$	$^{a}8.54^{b}$	$^{b}8.51^{b}$	$^{e}8.41^{b}$	$^{f}8.38^{b}$	٠.٠٢١
	٧	$^{d}8.49^{a}$	$^{c}8.50^{a}$	$^{a}8.56^{a}$	$^{b}8.54^{a}$	$^{e}8.43^{a}$	$^{e}8.43^{a}$	٠.٠.٠١
	١٠	$^{d}8.28^{d}$	$^{c}8.37^{d}$	$^{a}8.48^{d}$	$^{b}8.43^{d}$	$^{e}8.13^{d}$	$^{f}8.10^{d}$	٠.٠.٠١٢
	١٤	$^{d}8.12^{e}$	$^{c}8.24^{e}$	$^{a}8.40^{e}$	$^{b}8.33^{e}$	$^{e}8.05^{e}$	$^{f}8.01^{e}$	٠.٠١١
LSD%		٠.٠١٨	٠.٠٢٠	٠.٠١٧	٠.٠١٧	٠.٠١٣	٠.٠١٤	
Total Bacterial Counts (10^7×)	٠	$^{c}17.35^{d}$	$^{b}17.40^{c}$	$^{a}17.51^{d}$	$^{a}17.47^{d}$	$^{d}17.28^{c}$	$^{e}17.19^{c}$	٠.٠٤٣
	٣	$^{c}17.42^{b}$	$^{b}17.48^{b}$	$^{a}17.55^{c}$	$^{a}17.55^{b}$	$^{d}17.36^{b}$	$^{d}17.33^{b}$	٠.٠٣٩
	٧	$^{d}17.62^{a}$	$^{c}17.67^{a}$	$^{a}17.77^{a}$	$^{b}17.72^{a}$	$^{e}17.54^{a}$	$^{f}17.51^{a}$	٠.٠١١
	١٠	$^{c}17.37^{c}$	$^{c}17.36^{d}$	$^{a}17.60^{b}$	$^{b}17.52^{c}$	$^{d}17.00^{d}$	$^{e}16.92^{d}$	٠.٠١١
	١٤	$^{d}17.10^{e}$	$^{c}17.11^{e}$	$^{a}17.44^{e}$	$^{b}17.34^{e}$	$^{e}16.90^{e}$	$^{f}16.80^{e}$	٠.٠٤٠
LSD%		٠.٠١٥	٠.٠١٤	٠.٠٢١	٠.٠٢١	٠.٠٢٦	٠.٠٥٨	

■samples as in Table (3)
cfu :Colony Forming Unit N.D: Not Detected S.P: Storage Period
Means in a column) between times (not sharing the some superscript are significant different at p %٠.٠٥ >
Means in a raw) between treatments (not sharing the some superscript are significant different at p %٠.٠٥ >

Data for sensory evaluation of different Yoghurt sample fresh and during cold storage are shown in Table (7). It was clear that the addition of BSG up to 6% increased significantly the color, flavor, texture and overall acceptability of fresh Yoghurt. Addition of 8% %١٠ & BSG resulted in a marked decrease in sensory evaluation ,but the samples were still of an acceptable range. However, cold storage of Yoghurt samples affected the sensory evaluation of Yoghurt samples since a gradual decrease was observed in all treatments with cold storage elongation. Generally, all samples are accepted among the storage periods .Amaya Llanoa *et al* ,. (٢٠٠٨)

Sensorial testing showed that it is possible to produce Yoghurt with good functional and sensorial properties using hydrolyzed jicama starches (2.03g/100g) as a fat substituate. El-Nagga and Abd El–Tawab, (2012) reported that the addition 2% from dibs have a high values of sensorial properties. (Shori *et al*., 2013) founded thatfish collagen increased FAAs (Free amino acids) content in Yoghurt and the enhanced proteolysis of

certain milk proteins may be responsible for improving organoleptic properties of A. sativum-Yoghurt.

Table (7): Effect of BSG concentration on sensory evaluation of fresh and during stored Yoghurt samples

Properties	S.P Days	Treatments C	T1	T2	T3	T4	T5	LSD%
Color & Appearance (٢٠)	٠	$^{d}17.16^{a}$	$^{c}17.84^{a}$	$^{a}19.16^{a}$	$^{b}18.00a$	$^{e}13.84a$	$^{e}13.84^{a}$	٠.٠٣١
	٣	$^{d}17.16^{a}$	$^{c}17.34^{b}$	$^{a}18.16^{c}$	$^{b}17.36c$	$^{e}13.14^{b}$	$^{e}13.13^{b}$	٠.٠١٩
	٧	$^{c}16.46^{b}$	$^{c}16.50^{c}$	$^{a}18.50^{b}$	$^{b}17.50^{b}$	$^{d}13.13^{c}$	$^{e}13.10^{c}$	٠.٠٢٢
	١٠	$^{c}16.16^{c}$	$^{c}16.16^{d}$	$^{a}17.34^{d}$	$^{b}16.66^{d}$	$^{d}13.00^{d}$	$^{e}12.66^{d}$	٠.٠٢٤
	١٤	$^{d}15.34^{d}$	$^{c}16.00^{e}$	$^{a}16.84^{e}$	$^{b}16.66^{d}$	$^{e}12.84^{e}$	$^{e}12.42^{e}$	٠.٠٢٣
	LSD%	٠.٠١٨	٠.٠١٥	٠.٠١٧	٠.٠١٩	٠.٠١٥	٠.٠١٥	
Flavor (٥٠)	٠	$^{b}42.10^{a}$	$^{ab}42.50^{a}$	$^{a}45.50^{a}$	$^{b}44.15^{a}$	$^{c}35.85^{a}$	$^{c}34.15^{a}$	٠.٤٤٨
	٣	$^{c}42.10^{b}$	$^{c}42.10^{ab}$	$^{a}45.40^{a}$	$^{b}43.35^{b}$	$^{d}35.00^{b}$	$^{e}34.15^{a}$	٠.٠٢٠
	٧	$^{a}41.25^{b}$	$^{a}42.10^{ab}$	$^{a}42.90^{b}$	$^{a}42.90^{c}$	$^{b}35.00^{b}$	$^{c}33.75^{a}$	٠.٥٥٤
	١٠	$^{c}40.00^{b}$	$^{c}40.40^{b}$	$^{a}41.25^{c}$	$^{b}41.00^{d}$	$^{d}32.90^{c}$	$^{d}32.90^{a}$	٠.٠٢٠
	١٤	$^{c}40.00^{b}$	$^{c}40.40^{b}$	$^{a}41.20^{d}$	$^{b}40.85^{e}$	$^{d}32.90^{c}$	$^{e}32.50^{a}$	٠.٠٢٠
	LSD%	٠.٣٣٧	٠.٤١٧	٠.٠٥٥	٠.٠١٧	٠.١٥٢	٠.٦٢٣	
Body & Texture (٣٠)	٠	$^{a}27.99^{a}$	$^{ab}27.51^{a}$	$^{b}26.76^{a}$	$^{c}25.74^{a}$	$^{d}23.76^{a}$	$^{d}23.25^{a}$	٠.٢٦١
	٣	$^{a}26.25^{b}$	$^{a}26.25^{b}$	$^{a}26.25^{b}$	$^{b}25.26^{b}$	$^{c}23.49^{b}$	$^{d}22.26^{b}$	٠.٠٤٦
	٧	$^{a}26.25^{b}$	$^{b}25.74^{c}$	$^{c}24.99^{c}$	$^{d}24.75^{c}$	$^{e}23.49^{b}$	$^{f}21.75^{c}$	٠.٠٥٩
	١٠	$^{a}26.01^{b}$	$^{b}25.74^{c}$	$^{c}24.75^{d}$	$^{d}24.24^{d}$	$^{e}21.24^{c}$	$^{e}21.24^{d}$	٠.٠٢٠
	١٤	$^{a}25.50^{b}$	$^{b}25.26^{d}$	$^{c}24.24^{e}$	$^{c}24.24^{d}$	$^{d}21.24^{c}$	$^{e}20.76^{e}$	٠.٠٢٧
	LSD%	٠.٢٨٨	٠.٠١٧	٠.٠٣٢	٠.٠٣٤	٠.٠٢٥	٠.٠٣٤	
Overall (١٠٠)	٠	$^{d}87.25^{a}$	$^{b}87.85^{a}$	$^{a}90.81^{b}$	$^{c}87.89^{a}$	$^{e}73.45^{a}$	$^{f}71.24^{a}$	٠.٢٢
	٣	$^{c}85.51^{b}$	$^{b}85.69^{b}$	$^{a}90.42^{a}$	$^{c}85.97^{b}$	$^{d}71.63^{b}$	$^{e}69.54^{b}$	٠.١٨
	٧	$^{c}83.96^{c}$	$^{c}84.34^{c}$	a86.39c	$^{b}85.15^{c}$	$^{d}71.62^{b}$	$^{e}68.60^{c}$	٠.٢١
	١٠	$^{b}82.17^{d}$	$^{b}82.30^{d}$	$^{a}83.34^{d}$	$^{c}81.90^{d}$	$^{d}67.14^{c}$	$^{e}66.80^{d}$	٠.٢٠
	١٤	$^{d}80.84^{e}$	$^{c}81.66^{e}$	$^{a}82.28^{e}$	$^{b}81.75^{e}$	$^{e}66.98^{d}$	$^{f}65.68^{e}$	٠.١٨
	LSD%	٠.٥٠	٠.٣٠	٠.١٧	٠.١٩	٠.١٥	٠.١٨	

■samples as in Table (3)
Means in a column) between times (not sharing the some superscript are significant different at p %٠.٠٥ >
Means in a raw) between treatments (not sharing the some superscript are significant different at p %٠.٠٥ >

CONCLUSION

Brewers spent grain) BSG) by-product is a novel ingredient that can be successfully used in a functional yoghurt production. BSG is a good source of different compounds such as protein, fat, ash, dietary fiber, carbohydrates, phenols and flavonoids. Many of them have antioxidant activity. Significant differences in chemical, microbiological and sensory evaluation properties were observed due to addition of BSG .

Sensory evaluation of all treatments confirmed the possibility of use brewers spent grain as byproduct in the Yoghurt Manufacturing for his chemical composition and the vital importance. In addition, this product can be considered as a new product with functional properties and health benefits.

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الاستفاده من تفلة المولت في تصنيع الزبادي الوظيفي

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في هذه الدراسه تم استخدام تفلة المولت في تدعيم الزبادي وذلك من خلال احلاله مع اللبن بنسبة صفر, ٢, ٤, ٦, ٨ و ١٠%. وقد تم تقدير التركيب الكيميائي والمحتوي الفينولي ومحتوي الفلافينويدات في تفلة المولت. تم تقيم عينات الزبادي الناتجه كيميائيا وميكروبيولوجيا وحسيا وذلك في العينات الطازجه وكذلك خلال التخزين البارد علي درجة حراره ٤ درجه مئويه لمدة ٣, ٧, ١٠ و١٤ يوم. وقد أظهرت النتائج اختلافات كبيره في تقييم التركيب الكيميائي والميكروبيولوجي والحسي مع زيادة نسبة تفلة المولت المضافه في الزبادي. اضافة نسبة ١٠% من تفلة المولت اعطت اعلي قيمه في المواد الصلبه والبروتين والالياف والرماد وقيمة الرقم الهيدروجيني ولكنه ادي الي انخفاض الحموضه والعد الميكروبي والخصائص الحسيه.