

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, microbial count and sensory properties .

INTRODUCTION

Brewers' spent grain (BSG) is obtained from barley (*Hordeum Brew 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.* 2006). It can represent approximately 30% (w/w) of the starting malted grain (Townsend 1979) which makes BSG a readily available, high volume and low cost by-product within the brewing industry (Celus *et al.* 2006; Forssell *et al.* 2008; Treimo *et al.* 2009). 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.* 2010). 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.* 2002; Mussatto *et al.* 2006; and Mallouchos *et al.* 2007). There is approximately 3.4 million tons of BSG produced annually in the European Union according to Eurostat Data (Mussatto *et al.* 2006; and McCarthy *et al.* 2012). 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 β -glucan (Mussatto 2006; Tang *et al.* 2009). 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.* 1994; and Jacobs *et al.* 1998).

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'Connell 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 of Yoghurt 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-Akin and Akin, 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-Sharqia 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 37°C 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 60 mesh sieve and kept frozen in glass jars at -18 °C until used Charilaos *et al.* (2009). 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.* (1997).

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 μ l) was transferred into a test tube and the volume adjusted to 500 μ l with distilled water and oxidized with the addition of 250 μ l of Folin-Ciocalteu 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) per 100 g of sample.

The total flavonoid content was determined according to (Zilic *et al.* 2012). Briefly, 50 μ l of 5% NaNO₂ was mixed with 100 μ l of extract. After 6 min, 500 μ l of a 10% AlCl₃ solution was added. After 7 min, 250 μ l 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 \pm 1 °C). Spent grain was mixed with distilled water (1:1 ml) before pH measurement as outlined by AOAC, (2007).

Titration 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.* (1980).

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 $95 \pm 1^{\circ}\text{C}$ / 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 $42 \pm 1^{\circ}\text{C}$, and the prepared starter was added (3%). The samples were placed into 100 mL plastic cups. The cups were incubated at $42 \pm 1^{\circ}\text{C}$ until complete coagulation (3h). After fermentation, yoghurt samples were cooled to $4 \pm 1^{\circ}\text{C}$ 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 10^{-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	۷.۴۰	۲۰.۸۶	۴.۳۴	۱۲.۱۷	۳.۹۰	۵۱.۳۳
Cow's milk	۸۵.۷۵	۳.۸۵	۴.۶	.	۰.۹۷	۴.۸۳

*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, syringic 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	% ۱.۴۷
Total phenol (mg GAE/100g)	۱۰۲۶.۶۰
Total flavonoids (mg CE/100g)	۱۶.۲۰
Gallic acid (mg/100g)	۲۹.۹۶۴
Protochatchuic acid (mg/100g)	۳۹.۹۵۸
Syrngic acid (mg/100g)	۴۹.۲۰۹
Coumarin (mg/100g)	۱۷.۷۴۶
Cinnamic acid (mg/100g)	۴۹.۷۰۹
Ferulic acid (mg/100g)	۱۱۹۶.۰۱۵
Sinapic acid (mg/100g)	۱۲۷.۷۹۸

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* (۲۰۱۰) 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 (۲۰۱۲) 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* (2012) 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	14.70 ^a	3.80 ^u	4.7 ^a	0.00 ^e	0.90 ⁱ	0.40 ⁱ	27 ⁱ
T1	16.17 ^e	4.10 ^{bu}	4.7 ^a	0.20 ^u	1.01 ^e	7.10 ^e	00.97 ^e
T2	17.66 ^u	4.02 ^{bc}	4.7 ^a	0.47 ^c	1.07 ^u	7.00 ^u	80.93 ^u
T3	19.17 ^c	4.90 ^{ab}	4.7 ^a	0.72 ^u	1.12 ^c	7.83 ^c	110.43 ^c
T4	20.78 ^o	0.27 ^a	4.7 ^a	0.94 ^a	1.18 ^o	8.79 ^o	140.77 ^o
T5	22.19 ^a	0.72 ^a	4.7 ^a	1.20 ^a	1.20 ^a	9.00 ^a	170.39 ^a
SD%	0.200	0.389	N.S	0.149	0.19	0.209	13.040

■ 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 % . . . >

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* (2011) found that the addition of callus extract no significant effect among the experimental and control samples ,El-Nagga and Abd El-Tawab (2012) 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					LSD%	
	C	T1	T2	T3	T4		T5
0	^a 0.74 ^b	^{ab} 0.73 ^b	^{ab} 0.73 ^c	^{ab} 0.72 ^a	^{bc} 0.71 ^a	^c 0.69 ^a	0.022
3	^a 0.75 ^c	^a 0.74 ^{ab}	^a 0.74 ^c	^b 0.70 ^d	^{bc} 0.69 ^d	^c 0.68 ^{ab}	0.020
7	^a 0.75 ^c	^a 0.74 ^{ab}	^a 0.75 ^{bc}	^b 0.70 ^d	^{bc} 0.68 ^{bc}	^c 0.67 ^c	0.024
10	^a 0.76 ^u	^a 0.75 ^{au}	^a 0.77 ^{au}	^b 0.69 ^u	^{bc} 0.68 ^{bc}	^c 0.66 ^c	0.023
14	^a 0.79 ^a	^b 0.76 ^a	^{ab} 0.78 ^a	^c 0.69 ^b	^{cd} 0.67 ^c	^d 0.64 ^d	0.023
LSD%	0.019	0.021	0.024	0.022	0.018	0.020	

■ samples as in Table (3)

Means in a column) between times (not sharing the some superscript are significant different at p % . . . >

Means in a raw) between treatments (not sharing the some superscript are significant different at p % . . . >

The changes in pH values of Yoghurt samples are shown in Table (5). The pH values are 4.70, 4.71, 4.60, 4.59, 4.50 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* (2008) found that Yoghurt samples with hydrolyzed jicama starches added did not show significant differences. Sendra *et al* (2010) postulated that the pH values ranged from 4.58 to 4.71 with the addition 0.2 to 1g/10g from citrus fiber and it has insignificant effect. Karaaslan *et al* (2011) 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						LSD%
	C	T1	T2	T3	T4	T5	
0	^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
7	^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
14	^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
21	^a 4.41 ^a	^b 4.45 ^a	^b 4.53 ^a	^c 4.58 ^a	^b 4.66 ^a	^a 4.71 ^a	0.02
28	^a 4.40 ^a	^b 4.43 ^a	^b 4.51 ^a	^c 4.56 ^a	^b 4.64 ^a	^a 4.70 ^a	0.01
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 %0.05 >

Means in a row) between treatments (not sharing the some superscript are significant different at p %0.05 >

Brewers Spent Grain was free from microorganism, Coliform bacteria and Molds &Yeasts not detected. Our results are in agreement with James *et al* (2010).

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 probiotic 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						LSD%
		C	T1	T2	T3	T4	T5	
		Count (cfu /gm)						
Lactobacillus Count (10 ⁷ x)	•	^a 7.65 ^u	^b 7.67 ^u	^a 7.70 ^u	^b 7.68 ^e	^c 7.61 ^c	^d 7.58 ^c	•••1•
	∩	^a 7.68 ^c	^b 7.71 ^c	^a 7.80 ^u	^b 7.74 ^c	^c 7.65 ^u	^d 7.65 ^u	•••∩∩
	∪	^c 7.83 ^a	^b 7.87 ^a	^a 7.91 ^a	^b 7.88 ^a	^c 7.81 ^a	^d 7.78 ^a	•••∪
	1•	^a 7.79 ^u	^b 7.77 ^u	^a 7.82 ^u	^b 7.79 ^u	^c 7.57 ^u	^d 7.52 ^u	•••1•
	1∩	^d 7.68 ^c	^c 7.69 ^c	^a 7.74 ^c	^b 7.71 ^u	^c 7.55 ^e	^d 7.49 ^e	•••1∩
LSD%		•••∩∩	•••∩∩	•••∩∩	•••∩∩	•••∩∩	•••∩∩	
Streptococcus Count (10 ⁷ x)	•	^a 8.40 ^c	^b 8.43 ^c	^a 8.51 ^c	^b 8.49 ^c	^c 8.37 ^c	^d 8.31 ^c	•••1•
	∩	^a 8.44 ^u	^b 8.47 ^u	^a 8.54 ^u	^b 8.51 ^u	^c 8.41 ^u	^d 8.38 ^u	•••∩
	∪	^a 8.49 ^a	^b 8.50 ^a	^a 8.56 ^a	^b 8.54 ^a	^c 8.43 ^a	^d 8.43 ^a	•••∪
	1•	^a 8.28 ^u	^b 8.37 ^u	^a 8.48 ^u	^b 8.43 ^u	^c 8.13 ^u	^d 8.10 ^u	•••1•
	1∩	^d 8.12 ^e	^c 8.24 ^e	^a 8.40 ^e	^b 8.33 ^e	^c 8.05 ^e	^d 8.01 ^e	•••1∩
LSD%		•••1∩	•••∩∩	•••1∩	•••1∩	•••1∩	•••1∩	
Total Bacterial Counts (10 ⁷ x)	•	^c 17.35 ^u	^b 17.40 ^u	^a 17.51 ^u	^a 17.47 ^u	^u 17.28 ^u	^e 17.19 ^u	•••∩∩
	∩	^c 17.42 ^u	^b 17.48 ^u	^a 17.55 ^u	^a 17.55 ^u	^u 17.36 ^u	^u 17.33 ^u	•••∩∩
	∪	^a 17.62 ^a	^b 17.67 ^a	^a 17.77 ^a	^b 17.72 ^a	^e 17.54 ^a	^d 17.51 ^a	•••∪
	1•	^c 17.37 ^u	^b 17.36 ^u	^a 17.60 ^u	^b 17.52 ^u	^u 17.00 ^u	^e 16.92 ^u	•••1•
	1∩	^d 17.10 ^e	^c 17.11 ^e	^a 17.44 ^e	^b 17.34 ^e	^e 16.90 ^e	^d 16.80 ^e	•••1∩
LSD%		•••1•	•••1∩	•••∩∩	•••∩∩	•••∩∩	•••∩∩	

■ 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 %•••• >

Means in a raw) between treatments (not sharing the some superscript are significant different at p %•••• >

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% %1• & 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.* (2008)

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 substitute. 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 that fish 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						LSD%
		C	T1	T2	T3	T4	T5	
Color & Appearance (٢٠)	٠	^a 17.16 ^a	^c 17.84 ^a	^a 19.16 ^a	^b 18.00 ^a	^e 13.84 ^a	^e 13.84 ^a	٠.٠٣١
	٣	^a 17.16 ^a	^c 17.34 ^b	^a 18.16 ^c	^b 17.36 ^c	^e 13.14 ^d	^e 13.13 ^d	٠.٠١٩
	٧	^b 16.46 ^b	^c 16.50 ^c	^a 18.50 ^b	^b 17.50 ^b	^d 13.13 ^c	^d 13.10 ^c	٠.٠٢٢
	١٠	^b 16.16 ^c	^c 16.16 ^c	^a 17.34 ^b	^b 16.66 ^b	^d 13.00 ^c	^d 12.66 ^c	٠.٠٢٤
	١٤	^b 15.34 ^d	^c 16.00 ^b	^a 16.84 ^b	^b 16.66 ^b	^d 12.84 ^c	^d 12.42 ^c	٠.٠٢٣
	LSD%	٠.٠١٨	٠.٠١٥	٠.٠١٧	٠.٠١٩	٠.٠١٥	٠.٠١٥	
Flavor (٥٠)	٠	^a 42.10 ^a	^{ab} 42.50 ^a	^a 45.50 ^a	^a 44.15 ^a	^d 35.85 ^d	^d 34.15 ^d	٠.٤٤٨
	٣	^a 42.10 ^b	^{ab} 42.10 ^{ab}	^a 45.40 ^a	^a 43.35 ^b	^d 35.00 ^d	^d 34.15 ^d	٠.٠٢٠
	٧	^a 41.25 ^b	^{ab} 42.10 ^{ab}	^a 42.90 ^b	^a 42.90 ^b	^d 35.00 ^d	^c 33.75 ^d	٠.٥٥٤
	١٠	^b 40.00 ^b	^c 40.40 ^b	^a 41.25 ^b	^b 41.00 ^b	^d 32.90 ^d	^d 32.90 ^d	٠.٠٢٠
	١٤	^b 40.00 ^b	^c 40.40 ^b	^a 41.20 ^b	^b 40.85 ^b	^d 32.90 ^d	^d 32.50 ^d	٠.٠٢٠
	LSD%	٠.٣٣٧	٠.٤١٧	٠.٥٥٥	٠.٥١٧	٠.١٥٢	٠.٦٢٣	
Body & Texture (٣٠)	٠	^a 27.99 ^a	^{ab} 27.51 ^a	^b 26.76 ^a	^b 25.74 ^a	^d 23.76 ^d	^d 23.25 ^d	٠.٢٦١
	٣	^a 26.25 ^b	^a 26.25 ^b	^b 26.25 ^b	^b 25.26 ^b	^d 23.49 ^d	^d 22.26 ^d	٠.٠٤٦
	٧	^a 26.25 ^b	^b 25.74 ^b	^c 24.99 ^c	^c 24.75 ^c	^d 23.49 ^d	^d 21.75 ^c	٠.٠٥٩
	١٠	^a 26.01 ^b	^b 25.74 ^b	^c 24.75 ^c	^c 24.24 ^d	^d 21.24 ^d	^d 21.24 ^d	٠.٠٢٠
	١٤	^a 25.50 ^b	^b 25.26 ^b	^c 24.24 ^d	^c 24.24 ^d	^d 21.24 ^d	^d 20.76 ^e	٠.٠٢٧
	LSD%	٠.٢٨٨	٠.٠١٧	٠.٠٣٢	٠.٠٣٤	٠.٠٢٥	٠.٠٣٤	
Overall (١٠٠)	٠	^a 87.25 ^a	^b 87.85 ^a	^a 90.81 ^b	^b 87.89 ^a	^e 73.45 ^d	^d 71.24 ^d	٠.٢٢
	٣	^c 85.51 ^b	^b 85.69 ^b	^a 90.42 ^a	^b 85.97 ^b	^d 71.63 ^d	^d 69.54 ^d	٠.١٨
	٧	^c 83.96 ^c	^c 84.34 ^c	^a 86.39 ^c	^b 85.15 ^c	^d 71.62 ^d	^e 68.60 ^c	٠.٢١
	١٠	^b 82.17 ^d	^b 82.30 ^d	^a 83.34 ^d	^b 81.90 ^d	^d 67.14 ^c	^d 66.80 ^d	٠.٢٠
	١٤	^b 80.84 ^e	^c 81.66 ^c	^a 82.28 ^e	^b 81.75 ^e	^d 66.98 ^d	^d 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 row) 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.

REFERENCES

- Ainsworth, P. İbanoglu S.; Plunkett, A İbanoglu E. and Stojceska V. (2007). (Effect of brewers spent grain addition and screw speed on the selected physical and nutritional properties of an extruded snack. *Journal of Food Engineering*; 81: 702–709.
- Ajanaku, K.O.; Dawodu, F.A.; Ajanaku, C.O. and Nwinyi. O.C.(2011). Functional and nutritional properties of spent grain enhanced cookies. *American Journal of Food and Technology*; 6(9):763-771.
- Al-Rawy, K. M. and A. M. Khalaf-Allah (1980). *Design and Analysis of Agriculture Experimental*. Text book. El-Mousil Univ. press Ninawa, Iraq. Pp. 487.
- Amaya Llanoa, S.L; A.L. Martinez-Alegria; J.J. Zazueta-Morales; F. Martinez (2008). Acid thinned jicama and maize starches as fat substitute in stirred yoghurt. *Food Science and Technology*; 41(7):1274-1281.
- Aman, P.; J.X. Zhang; G. Hallmans and E. Lundin(1994). Excretion and degradation of dietary fiber constituents in ileostomy subjects consuming a low fiber diet with and without brewer's spent grain. *J Nutr* 124:359–63.
- AOAC (2007). Association of Official Analytical Chemists. *Official Methods of Analysis*. 18th Ed. Ch. 34.p: 72-80. Dairy products, subchapter 8: Gaithersburg, Maryland, USA.
- APHA (1992). American Public Health Association, *Compendium of methods for the microbiological examination of foods*. Inc. Washington, DC.499-503.
- Aportela, P. A.; M. E. Sosa-Morales and J. F. Velez-Ruiz (2005). Rheological and physicochemical behavior of fortified yoghurt, with fiber and calcium. *Journal of Texture Studies*; 36(3): 333–349.
- Bartolome', B.; M. Santos; J. J. Jimenez; J. M. Del Nozal and C. G. Cordove's (2002). Pentoses and hydroxycinnamic acids in brewer's spent grain. *Journal of Cereal Science*; 36:51–58.
- Celus, I.; K. Brijs and J. A. Delcour (2006). The effects of malting and mashing on barley protein extractability. *Journal of Cereal Science*; 44: 203–211.
- Charilaos, X.; Maria M.; Evangelos T. and Paul C. (2009). Factors affecting ferulic acid release from Brewer's spent grain by *Fusarium oxysporum* enzymatic system *Bioresource Technology*; 100: 5917–5921.
- Chaudhary, V. K. and F. E. Weber. (1990). Dietary fiber ingredients obtained by processing brewer's dried grain. *Journal of Food Science*; 55:551–553.
- Deeth, H.C. and A.Y. Tamime (1981). Yoghurt, nutritive and therapeutic aspects, *Journal of Food Protection* ;44: 78–86.
- Dello, S. M.; N. Bertola; M. Martino and A. Bevilacqua (2004). Influence of dietary fiber addition on sensory and rheological properties of yoghurt. *International Dairy Journal*;14(3):263–268.
- De Man, J.C.; Rogosa, M. and Sharp, M.E (1960). A medium for the cultivation of lactobacilli. *J. Appl. Bacteriol.*; 22:130.

- DeVrese, M.; A. Stegman; B. Richter; S. Fenselau; C. Laue and J. Scherezenmeir (2001). Probiotics – compensation for lactase insufficiency, *American Journal of Clinical Nutrition* 73 421–429.
- El - Nagga. E. A. and Y.A. Abd El-Tawab (2012). Compositional characteristics of date syrup extracted by different methods in some fermented dairy products, *Annals of Agricultural Science*; 57(1): 29–36.
- Forsell, P.; H. Kontkanen; H. A. Schols; S. Hinz; V. G. Eijnsink and J. Treimo (2008). Hydrolysis of brewers' spent grain by carbohydrate degrading enzymes. *Journal of the Institute of Brewing*;114:306–314.
- Garc-Lopez; E. Sayas-Pe, F. J.; E. Sendra; Y. Lario; J. Fernandez-a' Rheology of orange fiber .(٢٠٠٦) Alvarez -Barbera and J. A. Perez .٥٩-٥٥ "(١)١١ghurt. *Milchwissenschaft*; enriched yo
- Gibson G.R.; J.M. Saveedra; S. Mac-Farlane and G.T. Mac-Farlane (1997). Probiotics and intestinal infections, in: R. Fuller (Ed.), *Probiotic.2: Applications and Practical Aspects*, Chapman & Hall, NewYork, : 10–39.
- Guñ ler-Akin, B. and Akin, M.S.(2007). Effects of cysteine and different incubation temperatures on the microflora, chemical composition and sensory characteristics of bio-yogurt made from goat's milk.*Food Chemistry*; 100 (2):788–793.
- Hassanein, A.M. and Somaya, M. Moursy(2008). Production of yoghurt fortified by hull-Less barley flour. *Egypt. J. Agric. Res.*; 86(2):643-652.
- Hess, S. J.; R. F. Roberts and G. R. Ziegler (1997). Rheological properties of nonfat yoghurt stabilized using *Lactobacillus delbrueckii spp. bulgaricus* producing exopolysaccharides or using commercial stabilizer systems. *Journal of Dairy Science*; 80 (2): 252–263.
- Hsu, H. W.; L. D. Vavak; L. D. Satterlee and G. A. Miller (1977). Amultienzyme technique for estimating protein digestibility. *Journal of Food Science*;42:1269–1273.
- Imène Jaziri, A. A; A. M. Ben Stama; C. D. Houcine Mhadhbi; C. M. Urdaci and M. Hamdi (2009). Effect of green and black teas (*Camellia sinensis* L.) on the characteristic microflora of yoghurt during fermentation and refrigerated storage, *Food Chemistry* ;112 : 614–620.
- Jackson, M.S.; A.R. Bird and A.I. Mc-Orist (2002.) Comparison of two selective media for the detection and enumeration of lactobacilli in human faeces, *Journal of Microbiological Methods*; 51: 313–321.
- Jacobs, D.; K. Meyer; L. Kushi and A. Folsom(1998). Whole-grain intake may reduce the risk of ischemic heart disease death in post-menopausal women: the LoweWomen's Health Study. *Am J Clin Nut* 66:1264–76.
- James, A. R.; J.A. K. l'Anson; J. Treimo; B.C.Faulds; F. T. Brocklehurst; G.H. V.Eijnsink and W.K. Waldron (2010). Profiling brewers' spent grain for composition and microbial ecology at the site of production. *Food Science and Technology*; 43 :890–896
- Joo, S.J.; Choi, K.J.; Kim, K.S.; Lee, J.W. and Park, S.K.(2001). Characteristics of yoghurt prepared with 'jinpum' bean and sword bean (*Canavalin gladiata*). *International Journal of Postharvest Technology and Innovation*; 8:308–312.

- Karaaslan, M.; Mustafa O.; Hasan V. and Huseyin, T. (2011). Phenolic fortification of yoghurt using grape and callus extracts. *Food Science and Technology*; 44 : 1065-1072.
- Ling, E. R. (1963). A textbook of dairy chemistry. Vol. II, 3rd Ed., Chapman and Hall, Ltd. London.
- Mallouchos, A.; L. Georgios and K. Michael. (2007). A rapid microwave-assisted derivatization process for the determination of phenolic acids in brewer's spent grains *Food Chemistry*; 102:606–611.
- Marshall, V. (1986). The microflora and production of fermented milks, *Progress in Industrial Microbiology* 23 1–44.
- McCarthy, A. L.; C. Y. O'Callaghan; A. Connolly; C.O. Piggott; R.J. Fitzgerald and N.M. O'Brien. (2012). Phenolic extracts of brewers' spent grain (BSG) as functional ingredients – Assessment of their DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells. *J. of Food Chemistry*;134(2):641-646.
- Messer, J.W.; Behney, J. and Leudecke, L.O.(1985). *Standard Methods for the Examination of Dairy products*.14th Ed. Am. Publ.Health Assoc., Washington.
- Mussatto, S. I.; G. Dragone and I. C. Roberto. (2006). Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science*; 43:1–14.
- Nontasana, S.; Anuchita, M. and Sirirat D. (2012), Application of Functional Colorant Prepared from Black Rice Bran in Yoghurt, *APCBEE Procedia*; 2 :62 – 67.
- O'Connell, J. E. and P. F. Fox. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: a review. *International Dairy Journal*;11:103-120.
- Palmer, J. J. (2006). Chapter 12: what is malted grain? In. *How to brew* Boulder, Colorado, USA: Brewers Association.
- Park, Y.S.; Kim, Y.S. and Shin, D.W.(2003). Changes in physicochemical characteristics and microbial populations during storage of lactic acid bacterial fermented vegetable yogurt. *Food Science and Biotechnology*; 12: 654–658.
- Pearce, L.E. and Heap, H.A. (1974). *Town Milk*. J. New Zealand Milk Board, 22:18.
- Peng, Y.; M. Serra; D. S. Horne and J. A. Lucey. (2009). Effect of fortification with various types of milk protein on the rheological properties and permeability of nonfat set yoghurt. *Journal of Food Science*;74 (9):C 666–C673.
- Ramirez-Santiago C. -Ramos-Solis, L. -Lobato-Calleros, C. -Peña-Valdivia, C. -Vernon-Carter, E. J. -Alvarez-Ramírez, J. (2010). Enrichment of stirred yogurt with soluble dietary fiber from *Pachyrhizus erosus* L. Urban: Effect on syneresis, microstructure and rheological properties. *J.of Food Engineering*; 101: 229-235.
- SAS Statistical analysis Institute. (2000). *SAS User's Guide*, Version 4.02 . SAS Inst., CARY, N.C.

- Sendra, E.; V. Kuri; J. Fernandez-Lopez; E. Sayas-Barbera; C. Navarro and J. A. Perez-Alvarez. (2010). Viscoelastic properties of orange fiber enriched yoghurt as a function of fiber dose, size and thermal treatment. *Food Science and Technology*;43: 708–714.
- Shori A.B.; A.S. Baba and P.F. Chuah (2013). The effects of fish collagen on the proteolysis of milk proteins, ACE inhibitory activity and sensory evaluation of plain- and *Allium sativum*-yogurt. *Journal of the Taiwan Institute of Chemical Engineers*; 44: 701–706.
- Tang, D.; Gang-Ming, Y.; Yuan-Zhe, H.; Song-Qing, H.; Bing L.; Lin, L.; Hui-Ling, L. and Devajit B. (2009). Recovery of protein from brewer's spent grain by ultrafiltration. *Biochemical Engineering Journal* ;48 : 1–5.
- Temmerman R.; B. Pot; G. Huys and J. Swings. (2002) Identification and antibiotic susceptibility of bacterial isolates from probiotic products, *International Journal of Food Microbiology*; 81 : 1–10.
- Terzaghi, B.E. and Sandine, W.E. (1975). Improved medium for lactic streptococci and their bacteriophages. *Appl. Microbiol.*; 29:807.
- Townsley, P. M. (1979). Preparation of commercial products from brewer's waste grain and trub. *MBA Technical Quarterly*, 16, 130–134.
- Treimo, J.; B. Westereng; S. J. Horn; P. Forssell; J. A. Robertson and C. B. Faulds., *et al.* (2009). Enzymatic solubilization of brewers' spent grain by combined action of carbohydrates and peptidases. *Journal of Agricultural and Food Chemistry*; 57:3316–3324.
- Vasiljevic, T.; T. Kealy and V. K. Mishra. (2007). Effects of beta-glucan addition to a probiotic containing yoghurt. *Journal of Food Science*; 72(7): C405–C411.
- Zilic S.; A. Serpen; G. Akillioglu; M. Jankovic and V. Gökmen. (2012). Distribution of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. *Journal of Cereal Science*; 56:652-658.

الاستفادة من تفلّة المولت في تصنيع الزبادي الوظيفي

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في هذه الدراسة تم استخدام تفلّة 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 تفلّة المولت. تم تقييم عينات الزبادي الناتجة كيميائيا وميكروبيولوجيا وحسب ذلك في العينات الطازجه وكذلك خلال التخزين البارد على درجة حراره ٤ درجة مئوية لمدة ٣، ٧، ١٠، و ١٤ يوم. وقد أظهرت النتائج اختلافات كبيره في تقييم التركيب الكيميائي والميكروبيولوجي والحسي مع زيادة نسبة تفلّة المولت المضافه في الزبادي. اضافة نسبة ١٠% من تفلّة المولت اعطت اعلى قيمه في المواد الصلبه والبروتين والالياف والرماد وقيمة الرقم الهيدروجيني ولكنه ادي الي انخفاض الحموضه والعد الميكروبي والخصائص الحسيه.