International Journal of
Dairy Science

ISSN 1811-9743

Academic
Journals Inc.

www.academicjournals.com

International Journal of Dairy Science 10 (6): 266-277, 2015
ISSN 1811-9743 / DOI: 10.3923/ijds.2015.266.277

Quality of some Dairy By-products Supplemented with Wheat Germ as Functional Beverages

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ABSTRACT

The goal of this study is preparing nutritious beverages based on buffalo's Butter Milk (BM) or Sweet Whey (SW), as dairy by-products, supplemented with Wheat Germ (WG). The results indicated that, WG powder characterized by high levels of proteins (32.0%), dietary fiber (18.4%), essential amino acids (12.97%), total phenols (0.55 mg GAE/g) and total flavonoids (108.31 mg CT/100 g). The minerals content in WG powder were 44.2, 7.2, 275, 968, 1026, 14.0 and 91 for Ca, Fe, Mg, P, K, Zn and Se, respectively. Preliminary trails indicated that supplementation of SW or BM with 2.0% WG powder and 3.0% sugar were the best levels to be accepted as sensory properties. The SW or BM supplemented with 2.0% WG were higher in yellowish degree, apparent viscosity and antioxidant activity, but lower in lightness and greenish degree compared with non-supplemented. The increasing in antioxidant activity and apparent viscosity was more pronounced in BM supplemented with 2.0% WG powder. However, SW beverage supplemented with WG gained the higher sensory scores compared with other treatments.

Key words: Sweet whey beverage, butter milk beverage, wheat germ properties

INTRODUCTION

There are some dairy by-products which considered as disposal materials such as sweet whey and butter milk. These by-products have much biological and beneficial effect. The butter milk is the aqueous phase released during the churning of cream in butter manufacture. Butter milk is lower in fat than regular milk, but it is high in potassium, vitamin B_{12}, calcium and riboflavin as well as it is a good source of phosphorus. Butter milk is a dairy ingredient widely used in the food industry because of its emulsifying capacity and its positive impact on flavor. The industrial uses of butter milk are to prepare functional mixes for various foods, such as sauces, chips and chocolate products (Chandan, 1997). In the dairy industry, butter milk is used in cheese making (Joshi *et al*., 1994), formulation of ice cream (Chandan, 1997), yoghurt (Trachoo and Mistry, 1998) and in the manufacture of recombined milks (Singh and Tokley, 1990). In addition, butter milk consumption may be associated with reduced cholesterol concentrations through inhibition of intestinal absorption of cholesterol.

On the other hand, whey is also a by-product in the process of cheese production, the composition and characteristics of whey are depending on the technology of the product and on the quality of the used milk (Jelicic *et al*., 2008). Liquid whey consists approximately 93% of water and contains almost 50% of total solids present in the milk of which lactose is the main constituent,

while proteins represent less than 1% of total solids. In fewer amounts; also minerals and vitamins are present (Beucler *et al*., 2005). Production of whey-based beverages started in 1970's until today, a wide range of different whey beverages has been enveloped. One of the oldest whey beverages is Rivella from Switzerland. However, despite of all difficulties, fresh whey processing has proved to be the most economical technological solution.

Wheat germ is the nutrient-rich embryo of the wheat kernel, or seed and it constitutes approximately 2.5% of the total weight of the wheat kernel. It is a rich source in several minerals such as potassium, iron and zinc, as well as considered a source of essential amino acids and vitamins e.g., thiamin, riboflavin and niacin (Brandolini and Hidalgo, 2012). The wheat germ is characterized also by a palatable taste due to its high oil and sugar contents. Although plant proteins are generally considered of low biological value; the protein isolate from defatted wheat germ meal has been recognized as highly nutritional and utilized for some food products (Ge *et al*., 2002). The wheat germ proteins were reported to consist of 34.5% albumin as the major fraction followed by globulins (15%), gluten (10.6%) and prolamine (4.6%) and to have a very well balanced amino acids make-up being quite similar to that of egg proteins as indicated by Zhu *et al*. (2006). Also, although cereals, in general, are deficient in lysine, wheat germ is a rich source of lysine. The functional properties of wheat germ proteins, particularly their solubility and water retention, suggested their potential usage in food products (Vani and Zayas, 1995). Several potential health and therapeutic effects of wheat germ have been described were consumption of wheat germ had cholesterol lowering effect in human subjects (Ostlund *et al*., 2003). Also, consumption of wheat germ agglutinin containing foods had shown to be associated with significant reduction of diabetes (type 2), cardiovascular diseases and some types of cancer as well as long term weight management (Van Buul and Brouns, 2014). Therefore, the aim of this study was to evaluate the production of functional beverages from buffalo's sweet whey or butter milk supplemented with wheat germ as nutritional ingredient.

MATERIALS AND METHODS

Materials: Fresh buffalo butter milk and sweet whey were obtained from Faculty of Agriculture, Cairo University, Egypt. Fresh wheat germ (Sakha 93) was obtained from South Cairo Milling Company, Egypt. Wheat germ was finely milled by using home mixer before it is used. Commercial granulated sugar cane produced by Sugar and Integrated Industries Company was purchased from local market at Cairo, Egypt.

Methods

Experimental

Pre-experimental: Pre-treatment was designed to estimate the preferable ratio of Wheat Germ (WG) powder and sugar which will be used in preparing the beverages from dairy by-products. Five ratios of the WG powder (2, 4, 6, 8 and 10% w/v) and three ratios of sugar (2, 3 and 5% w/v) were applied to prepare the sweet whey and butter milk beverages. The sensory properties evaluation of the resultant beverages revealed that the best ratio of sugar was 3%, while the best ratio of WG was 2% for both dairy by-products (sweet whey and butter milk).

Preparation of beverages: Both Butter Milk (BM) and Sweet Whey (SW) samples were divided individually into two equal portions. The first batch supplemented only with 3% sugar as a

controls. The latter batches were supplemented with sugar and WG at the rate of 3 and 2%, respectively. All batches were well stirred and heated at 85°C for 5 min. The beverages were poured in sterilized dark bottles and stored at 5±2°C for 10 days. Three batches were conducted from each treatment.

Wheat germ analysis

Chemical analysis: Moisture, ash, total nitrogen and fat content of WG sample ere determined according to AOAC (2007). The crude protein content in WG was obtained by multiplying the percentage of TN by 5.7. Available carbohydrates were determined by di-nitro-salicylic acid method according to BeMiller (2010). Total Dietary Fiber (TDF) and Insoluble Dietary Fiber (IDF) were determined according to AOAC (2007). Soluble Dietary Fiber (SDF) was calculated by subtracting the IDF proportion from the TDF. The contents of calcium, iron, magnesium, phosphorus, potassium, zinc and selenium were determined using a Pye Unicom SP 19000 Atomic Absorption Spectroscopy, in Central Laboratory Services, National Research Center, Egypt as described by AOAC (2007).

Extraction of phenolic compounds: The WG sample was alkaline hydrolyzed according to Kim *et al*. (2006). Briefly, 10 g sample was placed in quick fit conical flask and 200 mL of 2 M NaOH was added. The flasks were flushed with N_2 and the stopper was replaced. The samples were shacked for 4 h at room temperature. The pH was adjusted to 2 with 6 M HCl. The samples were centrifuged at 5000 rpm for 10 min and the supernatant was collected. Phenolic compounds were extracted twice with 50 mL mixture of ethyl ether and ethyl acetate (1:1). The organic phase was separated and evaporated under vacuum at 45°C and the samples re-dissolved in 3 mL methanol.

Determination of total phenolic content: The total phenolic content of WG sample was determined according to the Folin-Ciocalteau 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 Na_2CO_3 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 microgram of Gallic Acid Equivalent (GAE) per gram of sample.

Determination of radical DPPH scavenging activity: Free Radical Scavenging Activity (RSA) was determined using the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH˙) according to Grzegorczyk *et al*. (2007). The final concentration was 50 μM for DPPH˙ and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min. Inhibition percentage of the DPPH free radical was calculated by the following equation:

$$RSA\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the treatment.

Determination of total flavonoid content: The total flavonoid content was determined according to Zilic *et al*. (2012). Fifty microliter of 5% $NaNO_2$ was mixed with 100 µL of extract. After 6 min, 500 µL of a 10% $AlCl_3$ 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 microgram of Catechin Equivalent (CE) per gram of sample.

Amino acids determination: Weight germ samples were hydrolyzed in sealed tubes with 10 mL HCl (6 N) for 24 h at 110°C in a sandy bath. The hydrolyzed samples were filtered with membrane filter and evaporated at 40°C in a rotary evaporator then dissolved in 1 mL distilled water and evaporated once again in order to remove the traces of the acid. The residue was reconstituted in 1 mL of demonized water then 20 µL was injected into the amino acid analyzer for determination of amino acid composition of each sample. The amino acids were separated on a cation exchanger resin column (150×2.6 mm i.d., No. 2619 resin) using citrate buffer at pH 2.2, a column temperature of 53°C, a flow rate of 0.225 mL min^{-1} and a postcolumn reaction with ninhydrin (0.3 mL min^{-1} ninhydrin flow rate) followed by a photometric detection at 570 nm.

Beverages analysis

Chemical analysis: Total solids, fat, total protein, ash and acidity contents of both BW and SW beverages were determined according to AOAC (2007). The pH value of was measured using a laboratory pH meter with glass electrode (Hanna, Instrument, Portugal).

Determination of color: The color of beverage samples was evaluated using a Spectro-Colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode. The color was expressed in terms of L, a and b.

Where:

L = Value represents darkness from black (0) to white (100)
a = Value represents color ranging from red (+) to green (-)
b = Value represents color ranging yellow (+) to blue (-)

Antioxidant activity: The antioxidant activity of beverage samples was determined by means of a calibration curve prepared with ascorbic acid and expressed as milligram of Ascorbic Acid Equivalent (AAE) per milliliter of sample (Grzegorczyk *et al*., 2007).

Viscosity: Apparent viscosity of beverage samples was determined using a Brookfield Synchro-Lectric viscometer (Model LVT; Brookfield Engineering Inc. Stoughton, MA). Readings were taken at the speed of 12-100 sec^{-1} using spindle -00 at 6°C for upward curve. Apparent viscosity was expressed as mill Pascal second (mPa sec).

Sensory properties: Eleven expert judges were selected from staff member of Department of Dairy Science, National Research Center, Egypt, to select the best ratio of WG and sugar which will be used in preparing the beverages from dairy by-products and evaluate the resultant beverages. Beverage samples were evaluated for taste (10 points), odor (10 points), color (10 points), fluid ability (10 points) and all acceptability (50 points).

RESULTS AND DISCUSSION

Wheat germ powder

Chemical properties of wheat germ: Table 1 reveals the contents of protein, fat, ash, crude fiber and total carbohydrates contents as well as moisture content in the Wheat Germ (WG). The WG was contained 32, 9.37, 4.74, 10.21, 42.22 and 11.50% for protein, fat, ash, crude fiber, total carbohydrates and moisture, respectively. These results are in agreement with those reported by Majzoobi *et al*. (2012) and Abd El-Hafez (2013), they reported that WG powder, contained 30% protein, 10% fat, 4.0% ash and 44% total carbohydrate. As shown in Table 2, the WG characterized by its higher content in potassium (1026 mg/100 g), phosphorus (968 mg/100 g), magnesium (275 mg/100 g), selenium (91 mg/100 g), calcium (44.2 mg/100 g), zinc (14.0 mg/100 g) and iron (7.2 mg/100 g). Similar observations were also found by Majzoobi *et al*. (2012), Ammar (2012) and Abd El-Hafez (2013). Therefore, supplementation of some dairy products with WG gives a rise to high levels of minerals, protein and fiber.

Total phenols, flavonoids and antioxidant activity of wheat germ sample: Antioxidant compounds in food play an important role as a health-protecting factor. Natural phenolic compounds exert their beneficial health effects mainly through their antioxidant activity (Fang *et al*., 2002). These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non radical species and breaking chains to prevent continued hydrogen abstraction from substances (Shahidi and Naczk, 2003). The results in Table 3 showed that the content of WG from total phenol content as Garlic Acid Equivalent (GAE) was 0.55 mg/100 g, which reflected that the WG had higher total phenolic content. The same trend was observed in total flavonoids as catching equivalent (CT) in wheat germ, where it was 108.31 mg CT/100 g.

The free radical scavenging (DPPH) action or β-carotene is known to be one of the mechanisms for measuring antioxidant activity. Table 3 shows the antioxidant activity against the DPPH radical. Free Radical Scavenging Activity (RSA) was 91.84%, which reflected that the WG had high antioxidant activity due to its content of tocopherols (Ford-Martin, 2005). In the present study, the authors attempted to assess the suitability of wheat germ utilization for improvement the quality and nutritive value of some dairy products such as beverages.

Table 1: Gross chemical composition of wheat germ powder sample

	Chemical composition (%)				
Sample	Moisture	Protein	Fat	Ash	Carbohydrate
Wheat germ	11.50	32.00	9.37	4.74	42.22

Table 2: Mineral contents of wheat germ powder sample

	Mineral contents (mg/100 g)						
Sample	Ca	Fe	Mg	P	K	Zn	Se
Wheat germ	44.2	7.2	275	968	1026	14.0	91.0

Ca: Calcium, Fe: Iron, Mg: Magnesium, P: Phosphorus, K: Potassium, Zn: Zinc, Se: Selenium

Table 3: Total phenols, total flavonoids and antioxidant properties of wheat germ powder

Sample	Total phenols (mg GAE/g)	Total flavonoids (mg CT/100 g)	RSA (%)
Wheat germ	0.55	108.31	91.84

GAE: Garlic acid equivalent, CT: Catching equivalent, RSA: Free radical scavenging activity

Table 4: Dietary fiber of wheat germ powder

	Dietary fiber (%)		
Sample	Soluble	Insoluble	Total
Wheat germ	2.8	15.6	18.4

Table 5: Amino acids percentages of wheat germ powder

Amino acids	Wheat germ powder (g/100 g)
Essential amino acids	
Arginine	2.80
Histidine	0.72
Isoleucine	1.10
Leucine	1.80
Lysine	2.10
Methionine	0.55
Phenylalanine	1.10
Threonine	1.30
Valine	1.50
Total	12.97
Non-essential amino acids	
Alanine	1.70
Aspartic acid	2.60
Cysteine	0.43
Glutamic acid	3.60
Glycine	1.80
Proline	1.20
Serine	1.20
Tyrosine	0.81
Total	13.34

Dietary fiber of wheat germ: Fiber is the structural part of plant foods which can not be digested by humans. The total fiber of a food consists of two types: Soluble fiber and insoluble fiber. The WG fiber consists of soluble dietary fiber (2.8%) and insoluble dietary fiber (15.6%) as shown in Table 4. Fiber is an important part of a healthy diet. It can help to prevent heart disease, diabetes, weight gain and some cancers and it can also improve digestive health.

Amino acid composition of wheat germ sample: Data in Table 5 indicate that wheat germ contains all amino acids presented in wheat flour (essential and non-essential amino acid). Wheat germ protein contains seventeen amino acids especially the essential amino acids lysine, leucine, arginine and threonine, in which many cereals are deficient and therefore, is a potential nutritious food supplement (Yiqiang *et al*., 1999). Real digestibility of most amino acids in wheat germs was similar or slightly higher than those reported for soybean meal (Gonzalez-Vega *et al*., 2011). Based on obtained results it is possible to recommend the use of wheat germs as an alternative protein source, originated form mill industry, in the diets of humans.

Sweet whey and butter milk beverages

Chemical properties: Table 6 presented the chemical composition of sweet whey and butter milk beverages supplemented with or without wheat germ. In general, total solids, total protein and ash contents were higher in beverages made from Butter Milk (BM) than those made from Sweet Whey (SW). Also, supplementation with WG caused pronounced increase in total solids, total protein and ash contents due to the higher solids and protein contents in WG (Table 1). Inversely, pH value was lower in beverages made BM than that made from SW (Table 7). Supplementation with WG caused slight decrease in pH value only at day 1 and 3 compared with that non-supplemented. Over storage, slight decrease was observed in pH value with the time increased.

Table 6: Chemical composition of sweet whey and butter milk beverages supplemented with or without wheat germ

	Beverage samples			
Items (%)	CSW	TSW	CBM	TBM
Total solids	9.90	12.30	13.80	16.20
Total protein	1.99	2.06	3.36	4.87
Fat	0.10	0.30	0.20	0.40
Ash	0.51	0.58	0.62	0.69

CSW: Sweet whey beverage without wheat germ, TSW: Sweet whey beverage with 2% wheat germ, CBM: Butter milk beverage without wheat germ, TBM: Butter milk beverage with 2% wheat germ

Table 7: pH value of sweet whey and butter milk beverages supplemented with or without wheat germ during storage period at 5±2°C for 10 days

	pH of beverages treatments			
Storage period (day)	CSW	TSW	CBM	TBM
1	6.82	6.77	5.78	5.75
3	6.80	6.72	5.74	5.72
7	6.66	6.70	5.68	5.68
10	6.64	6.64	5.55	5.52

CSW: Sweet whey beverage without wheat germ, TSW: Sweet whey beverage with 2% wheat germ, CBM: Butter milk beverage without wheat germ, TBM: Butter milk beverage with 2% wheat germ

Table 8: Color parameters of sweet whey and butter milk beverages supplemented with or without wheat germ during storage period at 5±2°C for 10 days

	Color parameters		
Beverage samples and storage period (day)	L	a	b
CSM			
1	74.91	-4.31	11.04
3	77.30	-4.68	14.13
7	73.22	-4.20	11.26
10	77.40	-4.34	12.03
TSM			
1	73.81	-1.94	22.52
3	74.61	-0.26	25.99
7	74.20	-1.39	24.25
10	75.25	-0.32	22.78
CBM			
1	84.88	-4.01	13.09
3	87.59	-3.04	16.11
7	86.68	-3.92	13.67
10	86.98	-3.53	15.99
TBM			
1	82.96	-1.46	16.04
3	83.44	-0.88	17.37
7	82.81	-1.39	16.29
10	83.86	-0.70	17.56

CSW: Sweet whey beverage without wheat germ, TSW: Sweet whey beverage with 2% wheat germ, CBM: Butter milk beverage without wheat germ, TBM: Butter milk beverage with 2% wheat germ, L: Darkness from black (0) to white (100), a: Color red (+) to green (-), b: Color yellow (+) to blue (-)

Color parameters: Due to the considerable influence of the color of the products on consumer acceptance, color measurement was done on the beverage samples. As shown in Table 8, control BM was more lightness (L), yellowish (b) and less greenish (a) compared with control SW. These results could be attributed to the BM had higher protein and phospholipids contents than SW. The interactions between the lipids and protein contents caused forming complexes and greater particle sizes, which increase the lightness degree. Morin *et al*. (2006) reported that BM has a high

Table 9: Antioxidant activity of sweet whey and butter milk beverages supplemented with or without wheat germ during storage period at 5±2°C for 10 days

	DPPH (%)			
Storage days	CSW	TSW	CBM	TBM
1	0.207	0.410	0.318	0.437
3	0.047	1.081	0.185	1.330
7	0.045	1.085	0.166	1.119
10	0.069	1.048	0.178	1.152

DPPH: 1, 1-Diphenyl-2-picryl-hydrazyl, CSW: Sweet whey beverage without wheat germ, TSW: Sweet whey beverage with 2% wheat germ, CBM: Butter milk beverage without wheat germ, TBM: Butter beverage with 2% wheat germ

content of fat globule membrane (rich in proteins and phospholipids). Supplementation with WG decreased both lightness and greenish degree in both SW and BM beverages, while increased the yellowish degree. However, storage period at 5±2°C for 10 days had no much effect on the color parameters of SW and BM beverages.

Antioxidant activity: The results in Table 9 show that the antioxidant activity was higher in Control BM (CBM) than that in Control SW (CSW). The BM is contains most of Milk Fat Globule Membrane (MFGM). The MFGM is rich in phospholipids, especially phosphatidylcholine, phosphatidylethanolamine and sphingomyelin, which act as antioxidants (O'Connell and Fox, 2000). Spitsberg (2005) reported that among the health beneficial components of the MFGM are cholesterolemia-lowering factor, inhibitor of cancer cell growth, inhibitor of some pathogenic bacteria and phospholipids as agents against colon cancer, gastrointestinal pathogens, depression and stress. Supplementation with 2% WG had significantly effect on the antioxidant activity of both SW and BM beverages. However, the higher antioxidant activity in TSW, CBM and TBM were observed at day 3. Thereafter, the time of storage had no much effect on the antioxidant activities of all beverage samples. However, the antioxidant activity of CSW beverage was higher at day 1 and decline thereafter.

Apparent viscosity: As shown in Fig. 1, the apparent viscosity of SW and BM beverages as dairy by-products supplemented with or without WG decreased with the increasing of the shear rate, which reflected that all the beverage solutions showed non-Newtonian and pseudoplastic behavior. The decreasing rate was more pronounced in beverage characterized with higher viscosity. Also, it's clear that, the viscosity of the CBM beverage was higher than that of CSW which could be related to the higher total solids and protein contents basically found in BM compared to SW. A similar, the viscosity of SW and BM beverages supplemented with WG was higher than that non-supplemented. The TBM had the highest viscosity compared with other treatments throughout storage period (Fig. 1a-d). The viscosity of the CBM was higher than that of TSW until day 10, thereafter, no much difference was found in viscosity between CBM and TSW. The viscosity of beverage solutions decreased in the order: TBM>CBM>TSW>CSW. Over storage, gradual increase in apparent viscosity along with the increase in storage time except CSW, gradual increase was observed until day 7 and decline thereafter (Fig. 1d).

Sensory evaluation: Sensory evaluation of SW and BM supplemented with or without WG during storage period at 4±2°C for 10 days are shown in Fig. 2a-e. The results reveled, there was no significant difference in the taste (Fig. 2a), odor (Fig. 2b), color (Fig. 2c) consistency (Fig. 2d) and overall acceptability (Fig. 2e) scores among CSW, TBM and TSW beverages throughout storage period. This means that, addition of WG to SW beverage had no adverse effects on the taste, odor,

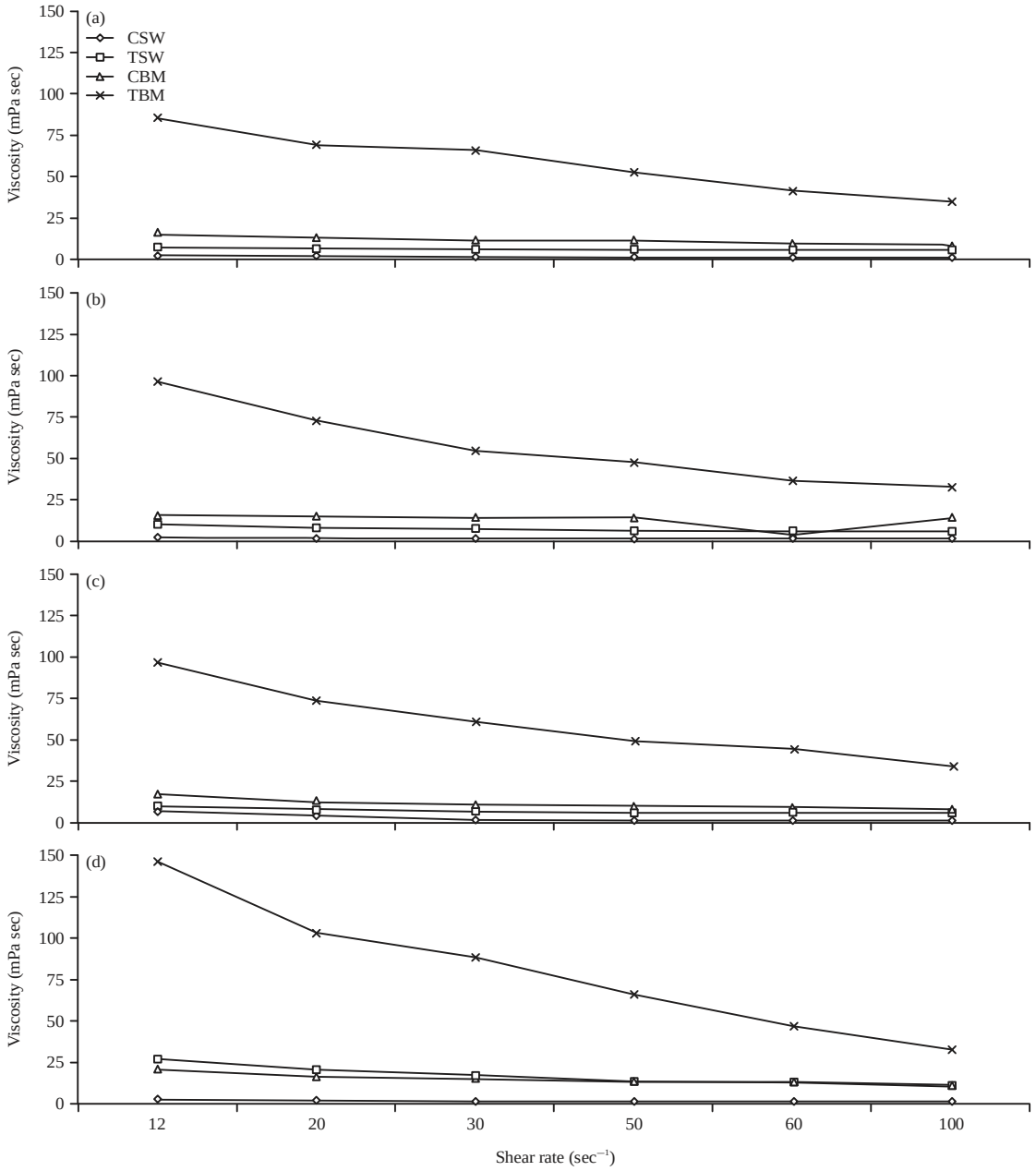

Fig. 1(a-d): Apparent viscosity of sweet whey and butter milk beverages supplemented with or without wheat germ during storage period at 5±2°C for (a) 1 day, (b) 3 days, (c) 7 days and (d) 10 days

consistency and overall acceptability scores. However, the TBM beverage gained the lowest sensory scores compared with other treatments. The lower sensory score of the TBM beverage could be attributed to the lower pH (Table 7) and higher viscosity (Fig. 1). Color scores had no significant affected by supplementation of SW or BM beverages with WG (Fig. 2c). Over storage at 5±2°C for 10 days, the sensory scores were stable in both SW and BM beverages until day 7 and decline thereafter.

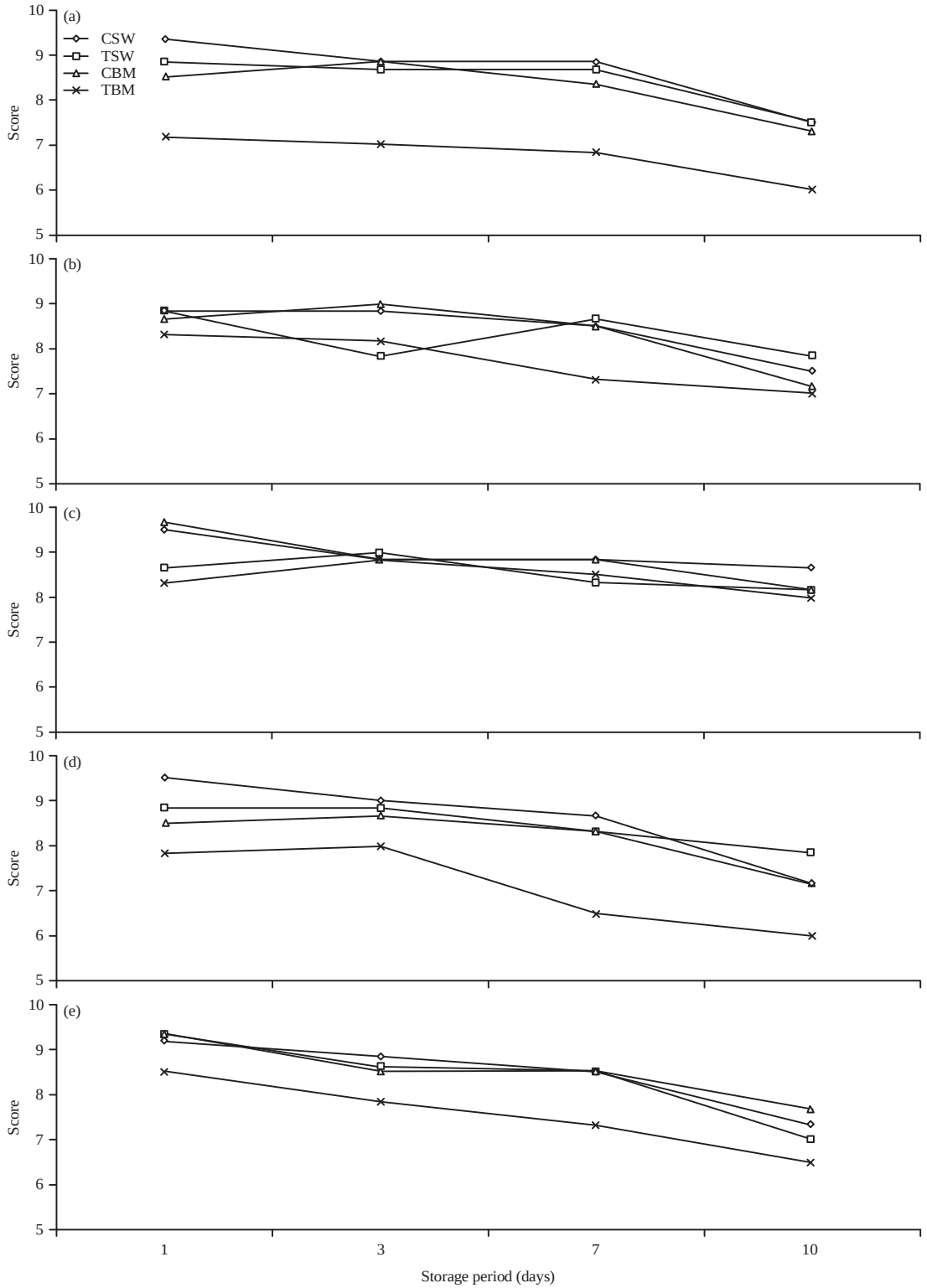

Fig. 2(a-e): (a) Taste evaluation, (b) Odor evaluation, (c) Color evaluation, (d) Consistency evaluation and (e) Overall acceptability evaluation of sweet whey and butter milk beverages supplemented with or without wheat germ during storage period at 4±2°C for 10 days

CONCLUSION

Wheat germ as a nutrient ingredient can be used in the supplementation of sweet whey or butter milk-by products for production functional beverages. Wheat germ supplementation at the ratio of 2% improved by-product beverages composition, antioxidant activity and rheological as well as sensory properties.

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