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Effect of Brine Solution Containing Ginger Extracts on the Properties of Egyptian White Brined Cheese

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ABSTRACT

The present study aims to investigate the physico-chemicals properties of white cheese pickled in brine solution containing ginger extracts. After cows milk coagulation and whey drained, fresh white cheese block was cut and divided into 3 equal portions. The first portion covered with 16.0% brine aqueous ginger extract and the second portion was covered with 16.0% brine ethanol ginger extract. The later was covered with 16.0% plain brine serves as a control. All white brined cheese treatments were stored at 5±2°C for the ripening period of 60 days. The results revealed that brine solution containing aqueous ginger extract or ethanol ginger extract enhances the growth of starter culture, protein proteolysis (WSN/TN), total volatile fatty acids (TVFAs), oxidative stability (reduced TBA) and sensory properties of white brined cheese compared with control cheese. In addition, brine solution containing aqueous ginger extract or ethanol ginger extract caused an increase in reddish and yellowish color degree and decrease in hardness of white cheese. However, most of these observations were more pronounced in white cheese pickled in brine aqueous ginger extract compared with that pickled in brine ethanol ginger extract; the differences were not significant.

Key words: Ginger extracts, Egyptian white brined cheese, physico-chemicals properties

INTRODUCTION

Cheese is a dairy product that has played an important role in human nutrition. Production of cheeses in Egypt rose from 293,000 t in 2000 to 620,000 t in 2012; almost all consumed locally (IDF., 2012). White cheeses are the most popular cheese in Egypt which produced by different procedures, traditional methods or ultrafiltration and stored at low temperature with or without brine and it is consumed either fresh or after pickling for few months. The brine solution plays an important role in cheese ripening, it has several purposes: Controlling microbial growth and enzyme activities, promoting curd syneresis, modifying flavor, texture and other physical properties (Guinee, 2004; Johnson *et al.*, 2009). Also, temperature of the brine solution and pH of the cheese can influence changes in weight and volume of cheese during brining (McMahon *et al.*, 2009).

Spices have already being used in the dairy food industry. Products like Jack cheese, garlic cheese and Cajun spice cheese among others are among consumer's favorites and all of them have something in common spices. Spices have been studied for their medicinal properties with antimicrobial, antiviral, antifungal and antiparasitic properties among them. Ginger (*Zingiber officinale*) is a nutritional complement and is on the FDA list of safe herbal preparations. It is also on the list of herbal drugs in the WHO monograph (WHO., 2002). Ginger has been used for thousands of years in several countries; including China as well as a remedy in Asian, Indian

and Arabic herbal traditions since long-ago (Ozgoli *et al.*, 2009). All ginger varieties contain an essential oil and a resin, collectively called an oleoresin. The exact composition of either depends on the variety of ginger, the method of drying, extraction and storage (Bone, 1997). Gingerols, shogaols, zingerone and paradol are the effective substances in ginger that have local effects on the digestive system (Ozgoli *et al.*, 2009). The 6-gingerol and 6-shogaol have antioxidant and anti-inflammatory, anticancer, antiemetic effect and can protect heart from blood clotting (Langner *et al.*, 1998; Craig, 1999; Mendi *et al.*, 2009). Ginger contains approximately 1.0-3.0% volatile oils and a number of pungent compounds (Chrubasik *et al.*, 2005). Also, gingerols are the most abundant pungent compounds in the fresh rhizome (Zick *et al.*, 2008). In addition, ginger extract was used to coagulate cow milk in which the milk clotting activity was caused due to the proteolytic activity of protease enzymes (Huang *et al.*, 2011; Hashim *et al.*, 2011). Further Hashim *et al.* (2011) revealed that ginger extracted enzyme had high specificity for β-casein followed by β-casein and α-casein and exhibited a similar affinity for α-casein, β-casein and β-casein. Its higher specificity for κ-casein with increasing temperature was also reported (Huang *et al.*, 2011). Therefore, the aim of this study was to investigate the feasibility of using brine solution containing ginger extracts for improving the biochemical, physical and sensory properties as well as the microbial load of Egyptian white brined cheese.

MATERIALS AND METHODS

Materials: Fresh cow's milk was obtained from the farm of Faculty of Agriculture, Cairo University, Egypt. The average milk composition was 87.67, 3.23, 3.40, 4.63 and 0.78% for moisture, total proteins, fat, lactose and ash, respectively. Fresh ginger rhizome was purchased from the local market at Cairo, Egypt. Calf rennet powder (HALA) and starter cultures (*Lactococcus lactis* ssp., *lactis* and *Lactococcus lactis* ssp. *cremoris*) were obtained from Chr. Hansen's Lab., A/S Copenhagen, Denmark. Sodium chloride was obtained from El-Naser Company of Alexandria, Egypt.

Methods

Ginger extract: Fresh ginger rhizome was washed for any contaminates, peeled, crushed finely and ground to paste in mortar. The ginger paste was divided into two equal portions: The first part (100 g) was soaked in 70% ethanol (1: 4) and kept for 3 days at 5±2°C. The extract was centrifuged at 5000×g for 30 min and this was repeated twice. The supernatant was collected and evaporated in a rotary vacuum evaporator (ROTAVAPOR R110, Buchi, Switzerland) at 50°C (Penna *et al.*, 2003). The concentrated ethanol extract was diluted with brine to obtained 1 L brine solution 16.0% NaCl (brine ethanol ginger extract). The second part (100 g) was soaked in 800 mL hot water (80°C) for 1 h, cooled to room temperature and kept for 3 days at 5±2°C. The aqueous extract, directly without any pretreatment was salted to obtained 1 L brine solution 16.0% NaCl (brine aqueous ginger extract).

Starter activation: Starter cultures were activated in maintenance broths (M 17) at 30°C for 24 h. After activation they were centrifuged (15000×g for 5 min at 4°C) in order to obtain pellet. Pellet was inoculated in 11% pasteurized skim milk powder for 24 h at 30°C.

Cheese making: White brined cheese was produced following the method of Sipahioglu *et al.* (1999). Fresh cow's milk (30 L) was preheated to 60°C and homogenized (Rannie, Copenhagen, Denmark) in two stages (13.6/3.5 MPa), followed by heated to 75°C and then cooled to 38°C. The

starter culture was added to cheese milk at rate 1% w/w then allowed ripening for 30 min and appropriate amount of rennet was added to achieve coagulation in 40 min. After coagulation, curd was cut into ~1 cm^3 cubes with vertical and horizontal knives. After being cut, the curd was left to settle for 10 min, transferred to mould and covered with cheesecloth. Additional cured was added to the mold as whey drained during the first 30-40 min. The curd mold was then stored at 21±3°C and turned every 6 h for 24 h. Cheese block was cut (7, 7 and 10 cm), divided into three equal portions and placed in three plastic containers. The first portion covered with 16.0% brine aqueous ginger extract (T_1) and the second portion was covered with 16.0% brine ethanol ginger extract (T_2). The third portion was covered with 16.0% plain brine (without ginger extract) serves as a control. All white brined cheese treatments were stored at 5±2°C for the ripening period of 60 days.

Microbiological analysis: Ten gram cheese sample was taken from cheeses at the age of 1, 15, 30 and 60 days and then homogenized in sterile 90 mL of 0.1% peptone water. Serial 8 fold dilutions in sterile 0.1% peptone water were prepared for bacterial analysis. The total viable bacterial counts were determined on plate count agar and incubated at 37°C for 24 h. M 17 agar was used for the enumeration of *Lactococcus* strains. Plates were incubated at 30°C for 24 h. Potato dextrose agar was used for yeast and mould enumeration. Plates were incubated at 25°C for 5 days (Marshall, 1992).

Chemical analysis: Moisture, fat and Total Nitrogen (TN) contents of cheese samples were determined according to AOAC (2007). The protein content was obtained by multiplying the percentage of TN by 6.38. Salt contents of white brined cheeses were estimated using Volhard method according to Richardson (1985). The pH value was measured using digital pH meter (HANNA, Instrument, Portugal) with glass electrode. The water soluble nitrogen (WSN/TN) ratio was estimated as described by Innocente (1997). The WSN/TN ratio was used as an index of proteolysis. Total volatile fatty acids (TVFAs) value was determined according to the method described by Koiskowski (1982). Values were expressed as milliliter of 0.1 N NaOH/10 g cheese. Thiobarbituric acid (TBA) was determined by the method of Pearson *et al.* (1981).

Texture profile analysis: Texture Profile Analysis (TPA) was performed on the cheese samples using the double compression test (TA-XT2 Texture Analyzer, Texture Technologies Crop, Scarsdale, NY) connected to a computer programmed with texture analysis software. An artificial plastic cylinder (45°Perspex Cone, 432-081) was attached to the moving crosshead. The crosshead speed was set at 70 mm min^{-1} in both upward and downward directions. The cheese sample was placed on a flat holding plate and the plastic cylinder inserted 20 mm below the cheese surface. Hardness, cohesiveness, springiness and gumminess were evaluated by TBA according to the definitions given by IDF (1991).

Color parameters: Hunter L, a and b parameters of cheese samples were measured 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 yellow (+) to blue (-)

Sensory properties: Eleven expert judges (males and females) were selected from staff member of Dairy Science Department National Research Center, Egypt, to evaluate the appearance, texture and flavor of the cheese samples. They scored the sample on the basis of nine-point hedonic scale, ranging from like extremely = 9 through like or dislike = 5 to dislike extremely = 1 as described by Piggott (1984). Cheese samples were cut into cubes (1.5×1.5×1.5 cm) and covered with plastic wrap to prevent dehydration. The cubes were coded with three-digit random numbers. Cheese samples were held at least 1 h at 20°C to equilibrate. Each judge was given three cubes of cheese per samples.

Statistical analysis: Data was expressed as Means±SE. Statistical analysis was performed using the GLM procedure with SAS (2004) software. Analysis of variance (ANOVA) and Duncan's multiple comparison procedure were used to compare the means. A probability of $p<0.05$ was used to establish statistical significance.

RESULTS AND DISCUSSION

Microbiological analysis: The changes in starter counts *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* ($\log_{10}$ CFU g^{-1}) of white cheese pickled in plain brine solution (C), brine aqueous ginger extract (T_1) or brine ethanol ginger extract (T_2) during storage at 5±2°C for 60 days are exhibited in Fig. 1a. The counts of starter culture were higher in T_1 and T_2 than in C and higher in T_1 than in T_2 at day 15 and 30; the difference was not significant ($p>0.05$). At day 60, the counts of starter culture were slightly higher ($p<0.05$) in T_2 compared with C and T_1. The increasing of the starter counts in T_1 and T_2 may be due to the ginger protease which could have provided the essential growth factors in the form of peptides and amino acids to improve the growth of the *Lactococcus* strains in the cheese (Adeniran *et al.*, 2010; Adesokan *et al.*, 2010). Such an effect has been reported by Abd El-Aziz *et al.* (2012) in ethanol ginger extract-fortified UF soft cheese and by Singh and Kumar (2013) in yoghurt containing ginger and mint extract. Over storage period, all white cheese samples showed slight increase in starter culture until day 15 ($p>0.05$), after which slight decreases were observed ($p>0.05$). The declines of starter culture in the end of storage period are probably because of the inhibitory effect of the high salt-in-moisture values of the cheese throughout maturation. Guinee and Fox (1987) reported that salting of cheese can influence the cheese pH due to its effect on microbial activity. Low levels of salt can stimulate bacterial activity; however, concentrations more than 2.5% have a negative effect.

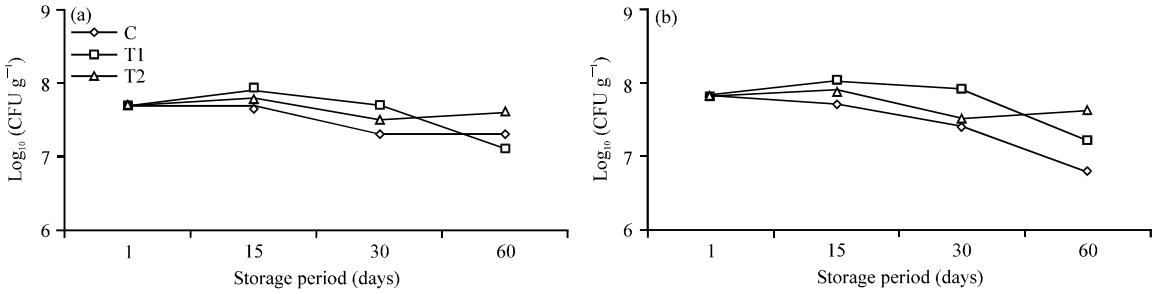

Fig. 1(a-b): (a) Starter culture and (b) Total counts of white cheese pickled in plain brine solution (c), Brine aqueous ginger extract (T_1) and brine ethanol ginger extract (T_2) at 5±2°C for 60 days

Table 1: Chemical composition of white cheese pickled in plain brine solution, brine aqueous ginger extract and brine ethanol ginger extract at 5±2°C for 60 days

Treatments and storage period (days)	Chemical composition (%)				
	pH	Moisture	Total proteins	Fat	Salt
C					
30	5.03±0.05[a]	55.07±0.63[b]	14.84±0.55[a]	22.58±1.50[a]	4.33±0.06[b]
60	4.92±0.10[ab]	59.15±1.41[a]	12.91±0.95[b]	20.75±1.78[a]	4.77±0.07[a]
T_1					
30	4.91±0.08[ab]	54.08±0.21[b]	15.56±0.36[a]	22.83±1.36[a]	4.39±0.11[b]
60	4.79±0.03[b]	58.19±2.08[a]	12.71±0.52[b]	20.50±1.75[a]	4.82±0.07[a]
T_2					
30	4.99±0.07[ab]	53.71±0.58[b]	15.80±0.55[a]	23.00±1.16[a]	4.31±0.14[b]
60	4.86±0.05[ab]	58.68±1.81[a]	12.59±0.63[b]	20.00±2.35[a]	4.86±0.18[a]

Means±SE, n = 3 with the same capital letters are not significantly different at ($p<0.05$), C: White cheese pickled in plain brine solution, T_1: White cheese pickled in brine aqueous ginger extract, T_2: White cheese pickled in brine ethanol ginger extract

A similar trend was observed in total counts of white brined cheese until day 30 (Fig. 1b). Thereafter, T_1 had the lowest total counts while the T_2 had the highest total counts. The counts of molds and yeasts were observed only in the end of storage period 5.5, 3.9 and 5.5 ($\log_{10}$ CFU g^{-1}) in C, T_1 and T_2, respectively. The lower counts of molds and yeasts in T_1 compared with C and T_2 might be due to the higher concentration of gingerols, shogaols, vitamin A and B, paradol and zingerine which act as antifungal compounds (Schulick, 1993; Kolapo *et al.*, 2007) in aqueous ginger extract.

Chemical changes: The changes in chemical composition of the white brined cheese during storage at 5±2°C for 60 days are presented in Table 1. Both T_1 and T_2 showed a slight decrease ($p>0.05$) in pH values at day 30 and 60 compared with C. A similar observation was found by Abd El-Aziz *et al.* (2012) in ethanol ginger extract-fortified UF soft cheese. The decrease in pH value attributed to ginger extract may enhance the growth of *Lactococcus* strains (Fig. 1a). However, pH values of all cheese treatments lies between the normal range (4.0-5.0) reported by Sipahioglu *et al.* (1999), Abd El-Salam and Alichanidis (2004) and Alichanidis and Polychroniadou (2008). pH values lower than 4.0 make the cheese very acid and may be brittle while pH values higher than 5.0 are not proper and safe for good keeping quality of white brined cheese (Abd El-Salam and Alichanidis, 2004). There was no significant difference ($p>0.05$) in moisture, total proteins, fat and salt contents among cheese treatments at day 30 and 60. However, moisture content of white cheese was significantly affected ($p<0.05$) by the time of storage. In particular, moisture content increased from 55.07, 54.08 and 53.71 to 60.45, 60.19 and 60.68 in C, T_1 and T_2, respectively, as storage period increasing from 30-60 days. As moisture content increased, total proteins and fat contents of white brined cheese decreased while salt content increased (Table 1).

As shown in Fig. 2a, type of brine solution affected protein proteolysis (WSN/TN ratio) of the white cheese. The WSN/TN ratio of T_1 and T_2 was higher than that of C, the difference being significant only at day 60 ($p<0.05$). On day 60, WSN/TN ratio of T_1 and T_2 were 21.43 and 20.36% higher than that of C (16.17%), respectively. However, the WSN/TN ratio of all cheese treatments lies in the normal range (10-25%) reported by Güven and Karaca (2001) and Abd El-Salam and Alichanidis (2004). The increase in proteolysis could be attributed to the proteolytic activity of the starter culture (Kaminarides and Stachtiaris, 2000) as well as proteolytic enzyme (zingibain) in

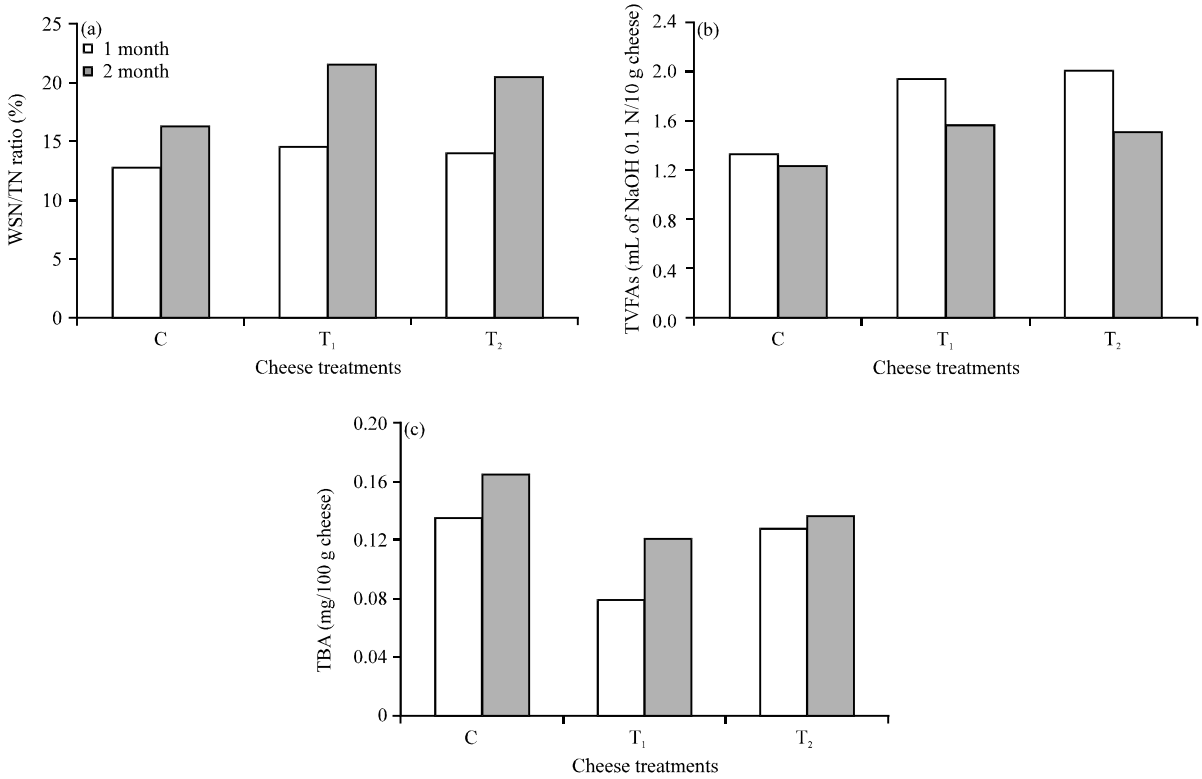

Fig. 2(a-c): (a) WSN/TN ratio, (b) TVFAs and (c) TBA of white cheese pickled in plain brine solution (C), brine aqueous ginger extract (T_1) and brine ethanol ginger extract (T_2)at 5±2°C for 60 days

ginger extract (Huang *et al.*, 2011; Hashim *et al.*, 2011; Thompson *et al.*, 1973). Alichanidis and Polychroniadou (2008) reported that proteinases and peptidases released by starter bacteria as well as by NSLAB are responsible for the production and accumulation of medium and small-size peptides and free amino acids during ripening and storage. Abd El-Aziz *et al.* (2012) have also found the effect of ginger extract on protein proteolysis was more pronounced in ginger extract-fortified UF soft cheese ($p<0.05$) at week 6. However, there was no significant difference in the WSN/TN ratio in T_1 and T_2 over storage period. These results indicating that both brine aqueous ginger and ethanol ginger extracts exhibited the same effect on the cheese proteolysis.

A similar, the TVFAs content of T_1 and T_2 was higher than that of C (Fig. 2b), the difference being significant only at day 30 ($p<0.05$). This could be attributed to a ginger extract has high content of essential oil which is a mixture of monoterpenic and sesquiterpenic compounds, contains the volatile compounds responsible for the characteristic ginger flavor (Zancan *et al.*, 2002). Chrubasik *et al.* (2005) reported that ginger contains approximately 1.0-3.0% volatile oils and a number of pungent compounds. The TVFAs content was similar in both T_1 and T_2 throughout the storage period. Over storage period, the TVFAs content showed a reduction at day 60 in all treatments compared with day 30. The changes in the TVFAs content during storage were found to be similar in ginger extract-fortified UF soft cheese (Abd El-Aziz *et al.*, 2012). The TBA content of T_1 was significantly lower ($p<0.05$) as compared to C and T_2 at day 30 and 60 (Fig. 2c). However, TBA content of T_2 was lower than that of C, the difference being significant only at day 60. The

antioxidant effect of ginger extract related to the presence of gingerol, shogaol, gingerdiol and curcumin in ginger rhizome (Zia-ur-Rehman *et al.*, 2003; Bandyopadhyay *et al.*, 2007). Zia-ur-Rehman *et al.* (2003) reported that the antioxidant effect depends on the variety of ginger, the method of extraction, storage temperature and storage time.

Texture profile: The changes in texture profile of the white cheese pickled in different brine solutions during storage at 5±2°C for 60 days are presented in Table 2. The results show that the hardness of T_1 and T_2 were lower as compared to the C, the difference being significant only between T_1 and C. The hardness of T_2 lies between the hardness of T_1 and C ($p<0.05$). The decrease in hardness of white cheese containing ginger extracts may be attributed to extended proteolysis that decreases the surface area occupied by the protein fraction in cheese microstructure, leading to a decrease of the force bearing component in cheese texture (Khosrowshahi *et al.*, 2006). Over storage period, all cheese treatments showed continued decrease in cheese hardness, the decrease rate was higher in both T_1 and T_2 than in C. However, no significant differences were observed in cheese cohesiveness, springiness and gumminess among C, T_1 and T_2 ($p>0.05$) at day 30 and 60, although the gumminess of T_1 and T_2 was lower than that of C ($p>0.05$). Also, continued decrease in cheese cohesiveness, springiness and gumminess were observed during storage period, the decrease being significant only in cheese cohesiveness and springiness ($p<0.05$).

Table 3 shows the changes in color degree of the white brined cheese during storage at 5±2°C for 60 days. There was no difference in whiteness degree of white brined cheese ($p>0.05$), even if whiteness degree of T_1 was numerically lower than that of C and T_2. However, the whiteness degree showed slight reduction in C ($p>0.05$) but remained more stable in T_1 and T_2 throughout the storage period. Yellowish degree of T_1 and T_2 was higher than those of C, the difference being significant only at day 30 ($p<0.05$). As storage periods increased; the yellowish degree of white brined cheese increased, the increasing was significant only in C treatment ($p<0.05$). Alichanidis and Polychroniadou (2008) reported the color of white brined cheeses range from off-white to yellow when they made from cow's milk. The reddish degree was significantly higher in T_1 than in T_2 and C ($p<0.05$) at day 30 and 60. Also, reddish degree was significantly ($p<0.05$) higher in T_2 than in C at day 60. Throughout the storage period, the reddish degree showed significant increasing in T_1 and T_2 ($p>0.05$) but remained more stable in C.

Table 2: Texture attributes of white cheese pickled in plain brine solution, brine aqueous ginger extract and brine ethanol ginger extract at 5±2°C for 60 days

Treatments and storage period (days)	Texture attributes			
	Hardness	Cohesiveness	Springiness	Gumminess
C				
30	6.22±0.49^{a}	0.65±0.01^{a}	0.74±0.01^{a}	4.41±0.51^{a}
60	5.68±0.33^{ab}	0.49±0.03^{b}	0.61±0.03^{b}	3.60±0.47^{a}
T_1				
30	4.98±0.30^{bc}	0.58±0.03^{a}	0.71±0.03^{a}	4.13±0.52^{a}
60	4.30±0.35^{c}	0.51±0.04^{b}	0.60±0.04^{b}	3.40±0.50^{a}
T_2				
30	5.80±0.36^{ab}	0.62±0.01^{a}	0.73±0.03^{a}	4.12±0.32^{a}
60	4.89±0.41^{bc}	0.50±0.01^{b}	0.61±0.02^{b}	3.32±0.43^{a}

Means±SE, n = 3 with the same capital letters are not significantly different at ($p<0.05$), C: White cheese pickled in plain brine solution, T_1: White cheese pickled in brine aqueous ginger extract, T_2: White cheese pickled in brine ethanol ginger extract

Table 3: Color degree of white cheese pickled in plain brine solution, brine aqueous ginger extract and brine ethanol ginger extract at 5±2°C for 60 days

Treatments and storage period (days)	Color degree		
	L	a	b
C			
30	88.8±2.98[a]	1.16±0.06[c]	5.27±0.23[c]
60	85.9±1.20[a]	1.18±0.01[c]	7.79±1.03[ab]
T_1			
30	86.6±1.40[a]	1.40±0.05[b]	7.55±0.81[ab]
60	87.7±1.25[a]	1.58±0.06[a]	7.89±0.65[a]
T_2			
30	88.4±1.99[a]	1.18±0.05[c]	6.81±0.32[b]
60	87.3±1.24[a]	1.35±0.10[b]	8.08±0.85[a]

Means±SE, n = 3 with the same capital letters are not significantly different at ($p<0.05$), C: White cheese pickled in plain brine solution, T_1: White cheese pickled in brine aqueous ginger extract, T_2: White cheese pickled in brine ethanol ginger extract, L: Darkness from black (0) to white (100), a: Color red (+) to green (-), b: Color yellow (+) to blue (-)

Table 4: Sensory properties of white cheese pickled in plain brine solution, brine aqueous ginger extract and brine ethanol ginger extract at 5±2°C for 60 days

Treatments and storage period (days)	Sensory properties		
	Appearance	Texture	Flavor
C			
30	7.45±0.20[a]	6.82±0.23[c]	7.09±0.21[c]
60	8.09±0.021[a]	7.45±0.21[b]	7.64±0.28[b]
T_1			
30	7.82±0.18[a]	7.64±0.15[ab]	7.73±0.12[ab]
60	7.91±0.16[a]	8.11±0.19[a]	8.19±0.21[a]
T_2			
30	8.10±0.25[a]	7.64±0.20[ab]	7.91±0.18[ab]
60	8.19±0.26[a]	7.73±0.30[ab]	8.02±0.31[ab]

Means±SE, n = 3 with the same capital letters are not significantly different at ($p<0.05$), C: White cheese pickled in plain brine solution, T_1: White cheese pickled in brine aqueous ginger extract, T_2: White cheese pickled in brine ethanol ginger extract

Sensory properties: Table 4 presents the sensory properties of the white cheese pickled in brine solution (C), brine aqueous ginger extract (T_1) or brine ethanol ginger extract (T_2) during storage at 5±2°C for 60 days. On day 30, the appearance, texture and flavor scores of C were lower than those of T_1 and T_2, the difference being significant only in texture and flavor scores ($p<0.05$). The higher flavor score of T_1 and T_2 may relate to the attractive smell of gingerol and other volatile oil of ginger (Bandyopadhyay *et al.*, 2007). Also, on day 60, T_1 and T_2 exhibited higher texture, (textural smoothing and softening) and flavor (more acceptable pungent flavor) scores than the corresponding control, the difference being significant only in flavor scores between T_1 and C ($p<0.05$). He *et al.* (1998) found that over storage or thermal processing, the gingerols may be modified to series of homologous compounds called shogaols which are more pungent than the gingerols.

CONCLUSION

Based on the finding of this study and the nutritional health properties of ginger as well as simple extraction, addition of the aqueous ginger extract to brine solution could be more suitable for improving the physicochemical properties of Egyptian white brined cheese.

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