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Original Article

Traditional Aroushe Cheese Enriched by Clove Essential Oil Embedded in Nanoencapsulation: A Novel Approach for Improving Physicochemical, Texture and Sensory Attributes

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ABSTRACT

Background and Objectives: Clove essential oil is used due to its biological activity related to human health (antimicrobial and antioxidant) and flavoring application to enrich traditional aroushe cheese embedded in nanoencapsulation.

Materials and Methods: Average particles size, polydispersity index and dynamic light scattering assessments were determined on nanoencapsulated clove essential oil (NCEO). Afterwards, the samples were fortified using Aroushe as a local cheese from Sangsar tribe in Semnan province, Iran, walnut powder (0, 1.9 and 3 % w/w) and NCEO (0, 0.3 and 0.6 % w/w), which physicochemical, rheology, microbial, sensory evaluation and microstructural characteristics were investigated during 40 days of shelf life in cold storage. Variance analysis was conducted for evaluation and the tests were performed in three repetitions.

Results: NCEO caused a decline trend in pH feature due to phenolic acids and antioxidant components as well as the highest and lowest moisture levels were reported for control and Aroushe cheese with 3 % walnut and 0.6 % NCEO (AWC_4) over 40 days. The most contents of fat and ash were obtained for AWC_4 and Aroushe cheese with 3 % walnut and 0.3 % NCEO (AWC_3) had the maximum protein. The microbial population was not distinguished in all treated samples on the 20^{th} days of shelf life and AWC_3 indicated the highest hardness, cohesiveness and springiness; nevertheless, AWC_4 had the greatest adhesiveness. The maximum score was attained for AWC_3 in overall acceptance of sensory evaluation and microstructure images prepared using a scanning electron microscopy exhibited that texture was uniform.

Conclusions: As a result, AWC_3 was an optimal selection for treated cheese sample and had the ability to apply as biofunctional natural products.

Keywords: Aroushe, Clove essential oil, Rheology, Nanoencapsulation, Walnut

Highlights

- In Sangsar region, Aroushe is prepared by roasting fresh sheep cheese with addition of turmeric and flour.
- Walnut powder and nanoencapsulated clove essential oil (NCEO) had improved some texture factors for cheese samples on account of protein and fat.
- NCEO caused a decline trend in pH feature due to phenolic acids and antioxidant components
- Cheese with 3 % walnut and 0.3 % NCEO was the most interesting and had necessary potential for industrial production and available to public.

Introduction

Cheese is a prominent product for many civilizations and manufactures in all over the world with a wide variety of flavors, textures and consumption patterns (1, 2). The main reason for cheese preparation was to obtain a product with extended shelf life compared to milk since ancient times; however nowadays, its manufacture with distinct organoleptic characteristics has become more prominent (3). Different cheeses are locally produced in Iran with unique attributes according to rheology properties, nutritional value and flavor and also the most traditional types include Liqhvan, Lor, Kouzeh, Pousti and Aroushe

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(4, 5). In Semnan province especially Sangsar region, Aroushe is prepared by roasting fresh sheep cheese with addition of turmeric and flour, which has particular nutritional interest.

The sustainable and beneficial products with rapid development in world population has elevated the application of animal foods enriched by plant sources for instance vegetables, fruits and seeds as antioxidant components (6-8). Since, free radicals play a major role in disease creation such as cancer, natural antioxidants are emphasized by supply food chain for a healthy life (2).

Essential oils (EOs) comprise distinct bioactive phytochemicals that improve nutritional value and flavor for product and also their nanoencapsulation as an effective technique is applied for enhancing stability, controlling release, preventing oxidation, anticancer activity, developing antimicrobial and antioxidant constituents (9). Clove (*Syzygium aromaticum* L.) is one of the important plant sources including phenolic components such as flavonoids, hydroxybenzoic, hydroxycinnamic, propene hydroxyphenyl, gallic and caffeic acids (10). Clove is an aromatic plant widely cultivated in tropical and subtropical countries, is not native to Iran (cultivated in Varamin, Tehran), rich in volatile compounds and antioxidants such as eugenol, β-caryophyllene, and α-humulene. CEO has received considerable interest due to its wide application in the perfume, cosmetic, health, medical, flavoring, and food industries. CEO has biological activity relevant to human health, including antimicrobial, antioxidant, and insecticidal activity; at least 30 compounds have been identified in CEO. Eugenol is the major compound, accounting for at least 50 %. The remaining 10 to 40 % is made up of eugenyl acetate, β-caryophyllene, and α-humulene. Less than 10 % correspond to minor or trace components such as diethyl phthalate, caryophyllene oxide, cadinene, α-copaene, 4-(2- propenyl)-phenol, chavicol, and α-cubebene (11). It has received much attention among other spices due to powerful antimicrobial and antioxidant attributes, which is useful for inhibiting various diseases owing to chemicals in high concentrations (10).

Nuts as plant sources form a significant section of daily diet; for instance, walnut with the scientific name *Juglans spp*. has 21 edible types and Iranian specie (*Juglansregia L.*) is known for seed production and extensively cultivated in the world (12). Walnut kernels contain 65 % fat including 7 % saturated, 20 % monounsaturated and 73 % polyunsaturated fatty acids and also rich in omega-6 and 3 unsaturated fatty acids, which are essential for dietary (13). These components play an important role for preventing heart diseases in walnut kernels, which have a high nutritional value due to micronutrients and protein structure (12).

Enrichment of various cheeses had been reported in previous studies such as ewe milk cheese comprising saffron (13), cheddar enriched with iron (13), processed cheese by tomato powder (13), Erzincan Tulum with cumin (13), gouda fortified using lavender flower powder (13), sheep cheese including chestnut tannin extract (13) and probiotic petit-suisse with distinct antioxidants including ascorbic acid, glucose oxidase, cysteine and jabuticaba extract (13) as well as use of clove EO (CEO) with salt water loaded into electrospun zein film for Iranian white cheese (14) and their chemical, microbiological, structural, textural, color and sensory features were investigated.

The characteristics of traditional cheeses had been comprehensively studied (5, 15); but until now, the technological and nutritional features as well as supplementation with plant-based materials such as cloves and walnuts for Aroushe have not been done. The aim of present research is to produce Aroushe formulated with walnut and nanoencapsulated clove EOs (NCEO).

Materials and Methods

Materials

Clove was purchased from Kermanshah province and walnut and also turmeric powder were obtained in local shops. Sheep milk was obtained from animal husbandry and pasteurized (in the pasteurizer at 63°C for 15 min); then, fungal rennet (rennilase) was prepared from DSM, France. Mold and yeast counts in glucose yeast extract chloramphenicol agar, violet red bile and brilliant green bile lactose broth were attained (Merck, Germany) and other chemicals with reagents in all analysis were of analytical grade.

Preparation of NCEO

The plant was thoroughly washed with distilled water, dried in the shade at room temperature, powdered using a laboratory mill, and the powder was passed through a 2 mm sieve. The CEO was extracted using a Clevenger device, which 50 g plant powder sample was distilled with water for 3 h. (9).

The combination of 10 g gum arabic and maltodextrin (similar levels) with 20 mL distilled water were mixed by a stirrer (Finetech, FTM-10, Korea) at 60 °C for 1 h and kept at 4 °C during 18 h to form nanocapsules. Then, 6 % (w/w) CEO solution with 2 % (w/w) tween 80 were added to prepared mixture and blended about 30 min; finally, samples were sonicated during 240 S at 24 kHz by ultrasonic homogenizer (sonicator**) (**Scientz**,** 750F, China) and freeze-dried (Martin christ, Alpha 1-2 LD Plus, Germany) for next analyses (20).

NCEO characteristics

The average particles size and polydispersity index (PDI) were determined by dynamic light scattering (DLS)

method (Zetasizer, Malvern, UK). The release assay of CEO from capsules in phosphate buffer solution (pH = 5.50) was performed through spectrophotometric detection for 20 days (every 5 days, once). The levels of 10 mg/mL NCEO and 10 mL buffer solution were added to centrifuge tubes and obtained mixtures were stirred using vortex (Genius 3, IKA Vortex, Germany). Afterwards, resulted samples were stored at 4 °C during shelf life and centrifuged; then, their supernatant was analyzed at 282 nm wavelength on different days and cumulative percentage of released CEO was calculated with the following Eq. 1 (16):

$$\text{Eq.1:Cumulative released EO (\%)} = [(\text{released level} - \text{EO amount at each sampling day}) / (\text{initial loaded} - \text{EO for treatment})] \times 100$$

Cheese processing

The temperature of 20 kg formulated milk was adjusted to 35 °C for enzyme and fungal rennet was blended with a little milk at 0.25 g/L; after that, obtained clot was stirred with whole mixture. When coagulation procedure completed about 60 min, several vertical and horizontal cuts were made in the scab using a blade. After 30 min, the dewatering operation was carried out through transferring the clot into a cloth bag and hung about 1 h to drain whey. At this stage, resulted clot was grated and slowly heated in a container during 4 h and also temperature was constantly controlled. The applied temperature started at 45 °C for clot and reached about 105 °C at the end of production; after 1 h stirring and heating, curd turned from a solid state into a liquid with a cut texture. At this step, white wheat flour (180 g) was added to harden tissue and turmeric (10 g) for improving color; when the time passed about 2 h, clot strengthened and oil came out and also separated. The cheese color changed from white to light brown and then became more colorful; immediately after production process, walnut powder (0, 1.9 and 3 %) as well as NCEO (0, 0.3 and 0.6 %) were added. Finally, Aroushe treatments were obtained with grainy texture and dark yellow color, which nine treatments were prepared.

Physicochemical compositions of produced cheese

A sample of grated cheese (10 g) was uniformly stirred (Nano technology researchers Company, HPMA 700, Iran) with distilled water (15 mL) for 15 min at ambient temperature and then pH was measured through a pH-meter (13). Moisture level was determined by drying procedure at 105 ± 1 °C in a 105°C oven (Behdad, Iran). and constant weight and also fat content was achieved regarding to international organization for standardization using Gerber-Van Gulik method (17). Protein was evaluated as total nitrogen using Kjeldahl method and then multiplied by the factor 6.38 to convert and also ash was measured with burning of samples at 550 °C in a muffle furnace (Labx, KSL-1400XS, USA) during 8 h (18).

Microbial evaluation

For microbiological investigation, 10 g of each treatment was collected under aseptic conditions and weighed in a sterile plastic bag; afterwards, samples were homogenized (APV, Denmark) with 90 mL sterile solution of 0.1% (w/v) peptone water. Coliform in violet red bile agar, *Escherichia coli* from brilliant green bile lactose broth (similarly at 30 ℃ for 48 h) and also mold and yeast counts in glucose yeast extract chloramphenicol agar (25 ℃ during 5 days) were cultivated. Microbiological data based on the logarithm number of colony forming units per g (log CFU/g) were reported (19).

Texture assessment

Samples with a suitable size were loaded on texture profile analysis (TPA, M350-10CT, Testometric Co., Ltd., Rochdale, Lancashire, England) through 40 mm diameter cylindrical probe and a test speed of 2.0 mm/s. A double cycle was programmed and the texture profile was determined using Texture Expert 1.05 software (Stable Microsystems). The basic properties of texture profile including hardness, adhesiveness, cohesiveness and springiness for treatments were investigated (20).

Rheological measurement

A controlled stress rheometer (Anton Paar, MCR301, Austria) was used to measure rheology of samples as well as in viscoelastic region, storage modulus (G′) and loss modulus (G″) were determined. The limits of linear viscoelastic behavior for samples were defined by applying 1 Hz frequency and strain elasticity (0.1 to 100 %) at room temperature conditions (18).

Sensory evaluation

The assessment was done in individual sensory booths consistent with ISO 8589 acceptable method. A trained descriptive sensory panel (40 women and men equally about 23 to 50 years old) rated cheese samples in triplicate using a flavor glossary and a global intensity scale of five-point hedonic, which smell, texture, taste, color and overall acceptance were assessed (18). According to Table 1, nine samples were analyzed in 40 days and A was considered as control.

Morphological properties

Microstructure of AWC_3 and A treatments was determined using SEM (Tescan, Model Mira3, Czech) technique (2).

Statistical analysis

In order to statistically analyze, the obtained data from different examinations were assessed with a factorial experiment in the form of a completely random design.

Firstly, achieved data and variance analysis were conducted for normality and evaluation, respectively. The tests were performed in three repetitions; then, the mean and standard deviation were attained. The multiple comparison means were carried out by Duncan test at 5 % significance level; in all stages, statistical analysis of data was done using SPSS statistics 22 software. A hedonic sensory evaluation of the samples was conducted were analyzed in score ranged from 1 (least pleasure) to 5 (best pleasure).

Results

NCEO characteristics

Fig. 1 depicts the graph related to NCEO by diameter and maximum number of particles are in 118 ± 4 nm range that indicates uniform distribution and appropriate size. The initial Polydispersity index was less than 0.3 for particles, which illustrated homogeneity structure as well as encapsulated forms were composed of small grains and many atoms located on surface. In this study, rapid release was observed in the 20 days, so that 70% of the EO was released during this period.

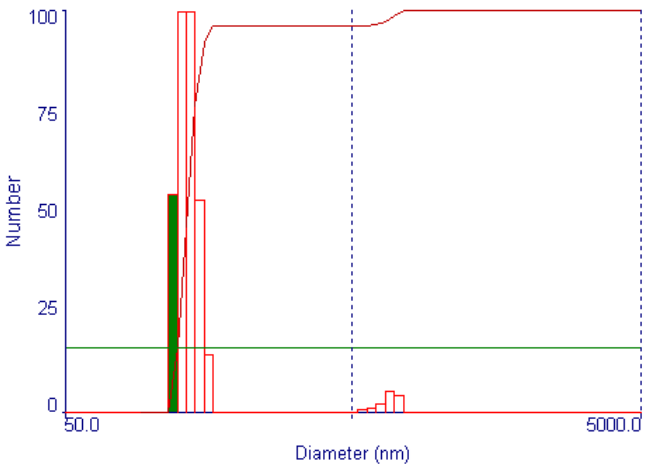


Fig. 1. Dynamic light scattering (DLS) curve of nanoencapsulated clove essential oil (NCEO) sample.

Compositional features of cheese treatments

The nine treatments were prepared according to formulation in Table 1. The chemical constituents of fortified cheese are listed in Table 2; generally, pH is reduced within 40 days for all treatments. On the 1st and 20th days, there was no significant difference among pH for samples, only this trend was observed in AC_7 (6.33 ± 0.01) and AWC_4 (6.48 ± 0.01) on the 20th day ($p < 0.05$). However, addition of walnut and NCEO to Aroushe cheese caused a significant difference about pH on the 40th day and the lowest (6.18 ±0.01) was reported for AC_8 and the highest (6.36 ± 0.013) in AWC_4 ($p < 0.05$). The walnut powders did not have a considerable effect on pH reduction, but NCEO led a decrease in this feature due to phenolic acids and antioxidants.

As depicted in Table 2, the most and least moisture were expressed for A and AWC_4 during shelf life as well as this level was considerably lowered in all samples; subsequently, AWC_4 demonstrated a noticeable reduction ($p < 0.05$). Arbutus extract at lower level (0.3 g/L) in cheese did not have a remarkable impact, but higher concentration (1 g/L) led to a decline moisture from 61 to 55 % (21).

Based on the obtained results (Table 2), fat content enhanced for all samples during storage and this level was less in A compared to others. The highest fat was detected for AWC_4 (32.75 ± 0.2 %) on the 40th day; in general, walnut and NCEO had a noticeable influence on this feature due to reason for the increased amount of fat in the samples containing walnut and NCEO was because of the higher fat in these compounds. The protein levels for A (28.9 to 27.65 %) and AWC_3 (37.1 to 32.57 %) were the least and most amount during shelf life, respectively; however, the difference was not significant between others in entire period ($p \geq 0.05$). Walnut and NCEO with small levels elevated the protein in samples and dry matter improved during ripening ($p < 0.05$). Also, ash in A sample was diminished, but this trend was enhanced in others and the most level for AWC_4 was obtained 4.93 ± 0.01 % on the 40th day.

Table 1. Various treatments of Aroushe cheese

coded	Skim milk	Cream (%w/w)	Flour	Walnut	NCEO	Turmeric	Rennet
A (Control)	90.5	5	3.5	0	0	0.66	0.2
AWC1	88.3	5	3.5	1.9	0.3	0.66	0.2
AWC2	88	5	3.5	1.9	0.6	0.66	0.2
AWC3	87.2	5	3.5	3	0.3	0.66	0.2
AWC4	86.9	5	3.5	3	0.6	0.66	0.2
AW5	88.74	5	3.5	1.9	0	0.66	0.2
AW6	87.64	5	3.5	3	0	0.66	0.2
AC7	90.34	5	3.5	0	0.3	0.66	0.2
AC8	90.04	5	3.5	0	0.6	0.66	0.2

A: Aroushe cheese; AWC_1: Aroushe cheese with 1.9 % walnut and 0.3 % NCEO; AWC_2: Aroushe cheese with 1.9 % walnut and 0.6 % NCEO; AWC_3: Aroushe cheese with 3 % walnut and 0.3 % NCEO; AWC_4: Aroushe cheese with 3 % walnut and 0.6 % NCEO: AW_5: Aroushe cheese with 1.9 % walnut: AW_6: Aroushe cheese with 3 % walnut; AC_7: Aroushe cheese with 0.3 % NCEO; AC_8: Aroushe cheese with 0.6 % NCEO.

Table 2. Changes in pH, moisture, fat, protein, and, ash of cheese samples at 1st, 20th, and 40th days of storage

Item	Time (day)	Treatments								
		A	AWC1	AWC2	AWC3	AWC4	AW5	AW6	AC7	AC8
pH	1	6.40 ± 0.01^{aA}	6.41 ± 0.02^{aA}	6.40 ± 0.01^{aA}	6.40 ± 0.01^{aA}	6.40 ± 0.01^{aB}	6.40 ± 0.01^{aB}	6.40 ± 0.01^{aA}	6.40 ± 0.01^{aA}	6.40 ± 0.01^{aA}
	20	6.43 ± 0.02^{bA}	6.42 ± 0.02^{bA}	6.43 ± 0.01^{bA}	6.40 ± 0.02^{bA}	6.48 ± 0.01^{aA}	6.44 ± 0.02^{bA}	6.38 ± 0.04^{bA}	6.33 ± 0.01^{cB}	6.40 ± 0.01^{bA}
	40	6.34 ± 0.01^{aB}	6.32 ± 0.01^{bB}	6.36 ± 0.01^{aB}	6.21 ± 0.01^{dB}	6.36 ± 0.013^{aC}	6.32 ± 0.01^{bc}	6.31 ± 0.01^{bB}	6.29 ± 0.01^{cC}	6.18 ± 0.01^{dB}
Moisture (%)	1	77.52 ± 0.1^{aA}	76.56 ± 0.3^{bA}	75.8 ± 0.1^{cA}	73.15 ± 0.1^{Ac}	74.8 ± 0.2^{dA}	75.29 ± 0.3^{cA}	74.47 ± 0.0^{dA}	75.1 ± 0.1^{cA}	75.68 ± 0.3^{cA}
	20	76.76 ± 0.5^{aB}	72.75 ± 0.3^{fB}	74.81 ± 0.1^{bB}	74.65 ± 0.5^{bB}	70.98 ± 0.0^{gB}	74.35 ± 0.0^{dB}	73.59 ± 0.2^{eB}	74.50 ± 0.0^{cB}	74.45 ± 0.0^{cB}
	40	75.45 ± 0.5^{aC}	72.01 ± 0.2^{eC}	74.18 ± 0.1^{bC}	72.45 ± 0.1^{eC}	69.28 ± 0.2^{fC}	72.81 ± 0.1^{dC}	73.01 ± 0.1^{cC}	72.97 ± 0.1^{dC}	73.22 ± 0.2^{eC}
Fat (%)	1	24.75 ± 0.2^{bB}	25.25 ± 0.2^{aC}	25.5 ± 0.5^{aC}	24.75 ± 0.2^{bC}	25.75 ± 0.2^{aC}	24.5 ± 0.5^{bB}	25.25 ± 0.4^{aC}	24.9 ± 0.1^{bC}	25.25 ± 0.3^{aB}
	20	25.5 ± 0.5^{dA}	27.75 ± 0.2^{aB}	26.25 ± 0.2^{cB}	27.50 ± 0.5^{aB}	27.75 ± 0.2^{aB}	26.75 ± 0.2^{bA}	27.75 ± 0.2^{aB}	25.75 ± 0.2^{dB}	25.75 ± 0.2^{dB}
	40	25.5 ± 0.5^{gA}	29.75 ± 0.2^{cA}	28.25 ± 0.2^{dA}	30.25 ± 0.21^{bA}	32.75 ± 0.2^{aA}	26.75 ± 0.2^{eA}	28.5 ± 0.5^{dA}	26.0 ± 0.01^{fA}	26.25 ± 0.2^{fA}
Protein (%)	1	28.9 ± 0.5^{hA}	29.88 ± 0.7^{gA}	33.89 ± 0.1^{cA}	37.1 ± 0.4^{aA}	34.58 ± 0.6^{bA}	30.05 ± 0.5^{fA}	33.80 ± 0.1^{cA}	32.81 ± 0.6^{dA}	31.07 ± 0.1^{eA}
	20	28.18 ± 0.8^{gA}	29.75 ± 0.2^{eA}	32.03 ± 0.2^{bB}	36.55 ± 0.1^{aB}	31.56 ± 0.4^{cB}	28.73 ± 0.2^{fB}	32.06 ± 0.1^{bB}	32.10 ± 0.6^{bA}	30.8 ± 0.1^{dB}
	40	27.65 ± 0.3^{gB}	28.63 ± 0.0^{fB}	30.84 ± 0.1^{dC}	32.57 ± 0.2^{aC}	30.7 ± 0.4^{dC}	30.93 ± 0.0^{dC}	31.90 ± 0.1^{bC}	31.90 ± 0.1^{bA}	29.84 ± 0.3^{eC}
Ash (%)	1	3.68 ± 0.03^{bA}	3.25 ± 0.07^{cC}	3.65 ± 0.01^{bB}	3.24 ± 0.06^{cC}	4.49 ± 0.04^{aC}	3.53 ± 0.07^{bA}	3.61 ± 0.02^{bC}	3.50 ± 0.08^{bA}	3.63 ± 0.06^{bB}
	20	3.61 ± 0.01^{bB}	3.31 ± 0.01^{eB}	3.66 ± 0.01^{bB}	3.46 ± 0.01^{dB}	4.79 ± 0.01^{aB}	3.49 ± 0.01^{cB}	3.66 ± 0.01^{bB}	3.50 ± 0.00^{cA}	3.68 ± 0.01^{bB}
	40	3.58 ± 0.03^{cC}	3.49 ± 0.02^{dA}	3.73 ± 0.04^{bA}	3.80 ± 0.02^{bA}	4.93 ± 0.01^{aA}	3.54 ± 0.01^{cA}	3.72 ± 0.01^{bA}	3.70 ± 0.04^{bA}	3.75 ± 0.06^{bA}

A: Aroushe cheese; AWC_1: Aroushe cheese with 1.9 % walnut and 0.3 % NCEO; AWC_2: Aroushe cheese with 1.9 % walnut and 0.6 % NCEO; AWC_3: Aroushe cheese with 3 % walnut and 0.3 % NCEO; AWC_4: Aroushe cheese with 3 % walnut and 0.6 % NCEO: AW_5: Aroushe cheese with 1.9 % walnut: AW_6: Aroushe cheese with 3 % walnut; AC_7: Aroushe cheese with 0.3 % NCEO; AC_8: Aroushe cheese with 0.6 % NCEO ($^{a-h}$: significant difference in row, $^{B-C}$: significant difference in each column).

Microbial assessment

Cheese samples were examined for coliform, *Escherichia coli*, mold and yeast during the 40th day of shelf life. Based on microbial results (Table 3), no growth was observed in all treatments on the 1st and 20th days. The lowest and highest populations for coliform, *Escherichia coli*, mold and yeast were obtained for AC_8 (0.1 ± 0.001, 0.2 ± 0.06 and 1 ± 00 log CFU/g) and also AW_6 (1.85 ± 0.01, 1.73 ± 0.02 and 12 ± 1 log CFU/g) on the 40th day, respectively. Moreover, microorganism number in A sample was more than AWC_1, AWC_2, AWC_3, AWC_4, AC_7 and AC_8.

Texture analysis

The texture parameters including hardness, adhesiveness, cohesiveness and springiness for cheese samples are reported in Table 4. The lowest level was expressed for A (0.56 ± 0.02 N) and AC_8 (0.56 ± 0.01 N) on the 1st day and the highest was represented in AWC_3 (1.687 ± 0.1 N) on the 40th day.

The most adhesiveness (AWC_4, 0.254 ± 0.02 N) was found in treatment, which had the maximum fat and the minimum water on the 40th day (Table 4). Adhesiveness had increased over time because of elevated fat; therefore, sample A indicated the least trend (0.047±0.03 N) on the 1st day; however, moisture was reduced.

Cohesiveness was elevated by higher protein and AWC_3 on the 1st day (0.77 ± 0.003) as well as A on the 40th day (0.51 ± 0.01) had the most and least levels, respectively. The mentioned attribute enhanced and strengthened with more fat and this ability returned to original state after removing the pressure. Since, fat level had elevated in samples over time, the springiness factor was developed; so, the highest value was obtained for AWC_4 (0.86 ± 0.01) on the 40th day and the lowest was detected in A (0.31 ± 0.02) on the 1st day.

Microbes (logCFU/mL)	Time (day)	Treatments								
		A	AWC1	AWC2	AWC3	AWC4	AW5	AW6	AC7	AC8
Coliform	1	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}
	20	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}
	40	1.75 ± 0.01^{bA}	0.90 ± 0.07^{dA}	0.95 ± 0.07^{dA}	0.50 ± 0.01^{eA}	0.25 ± 0.02^{bA}	1.25 ± 0.03^{cA}	1.85 ± 0.01^{aA}	0.54 ± 0.07^{eA}	0.10 ± 0.00^{fA}
E.coli	1	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}
	20	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}
	40	1.50 ± 0.01^{bA}	0.70 ± 0.02^{dA}	0.65 ± 0.07^{dA}	0.30 ± 0.01^{eA}	0.15 ± 0.01^{fA}	1.11 ± 0.01^{cA}	1.73 ± 0.02^{aA}	0.54 ± 0.04^{dA}	0.20 ± 0.06^{gA}
Mold &Yeast	1	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}
	20	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}	ns^{aB}
	40	7 ± 1^{bA}	5 ± 1^{cA}	3 ± 1^{cA}	4 ± 1^{cA}	2 ± 0^{dA}	9 ± 2^{bA}	12 ± 1^{aA}	2 ± 00^{dA}	1 ± 00^{eA}

Table 4. Comparison of average effects of Aroushe chesses supplemented with different walnut and NCEO on 1st, 20th, and 40th days of storage on texture (hardness, cohesiveness, adhesiveness and springiness) of samples

Parameters	Time (day)	A	AWC1	AWC2	AWC3	AWC4	AW5	AW6	AC7	AC8
Hardness (N)	1	0.560 ± 0.02^{dB}	0.592 ± 0.01^{cB}	0.634 ± 0.08^{cC}	1.201 ± 0.34^{aC}	0.873 ± 0.014^{bB}	0.598 ± 0.01^{cA}	0.610 ± 0.02^{cC}	0.590 ± 0.01^{cB}	0.56 ± 0.01^{dB}
	20	0.600 ± 0.01^{aA}	0.611 ± 0.02^{aB}	0.724 ± 0.05^{cB}	1.537 ± 0.02^{aB}	1.060 ± 0.03^{bA}	0.609 ± 0.38^{aA}	0.687 ± 0.02^{dB}	0.618 ± 0.02^{aB}	0.59 ± 0.01^{aA}
	40	0.618 ± 0.01^{aA}	0.674 ± 0.02^{dA}	0.765 ± 0.01^{cA}	1.687 ± 0.05^{aA}	1.110 ± 0.06^{bA}	0.624 ± 0.02^{aA}	0.762 ± 0.02^{cA}	0.662 ± 0.03^{dA}	0.61 ± 0.02^{aA}
Adhesiveness (N)	1	0.047 ± 0.003^{aC}	0.037 ± 0.004^{fC}	0.046 ± 0.001^{eC}	0.057 ± 0.03^{dC}	0.171 ± 0.06^{aC}	0.150 ± 0.02^{bC}	0.120 ± 0.03^{cC}	0.110 ± 0.02^{cB}	0.160 ± 0.05^{bB}
	20	0.083 ± 0.002^{fB}	$0.0411g\pm0.001^{hB}$	0.054 ± 0.005^{gB}	0.106 ± 0.031^{eB}	0.252 ± 0.02^{aB}	0.210 ± 0.04^{bB}	0.230 ± 0.02^{bB}	0.127 ± 0.01^{dB}	0.151 ± 0.01^{cB}
	40	0.090 ± 0.002^{gA}	0.0489 ± 0.002^{hA}	0.061 ± 0.002^{eA}	0.159 ± 0.04^{fA}	0.33 ± 0.01^{aA}	0.310 ± 0.01^{bA}	0.290 ± 0.01^{cA}	0.230 ± 0.02^{eA}	0.260 ± 0.02^{dA}
Cohesiveness (-)	1	0.56 ± 0.02^{hA}	0.65 ± 0.00^{fA}	0.69 ± 0.00^{eA}	0.77 ± 0.01^{aA}	0.73 ± 0.00^{dA}	0.66 ± 0.01^{gA}	0.65 ± 0.00^{fA}	0.74 ± 0.00^{cA}	0.76 ± 0.00^{bA}
	20	0.55 ± 0.00^{fB}	0.61 ± 0.03^{eB}	0.67 ± 0.00^{dB}	0.76 ± 0.00^{aB}	0.71 ± 0.01^{cB}	0.64 ± 0.02^{dB}	0.61 ± 0.02^{eB}	0.72 ± 0.02^{cA}	0.75 ± 0.01^{bB}
	40	0.51 ± 0.01^{eC}	0.59 ± 0.02^{dB}	0.65 ± 0.02^{cC}	0.75 ± 0.00^{aC}	0.69 ± 0.02^{cB}	0.64 ± 0.00^{bB}	0.57 ± 0.02^{dC}	0.68 ± 0.02^{cB}	0.70 ± 0.02^{bC}
Springiness (-)	1	0.31 ± 0.01^{gB}	0.39 ± 0.01^{fB}	0.57 ± 0.02^{dC}	0.65 ± 0.02^{bB}	0.73 ± 0.03^{aC}	0.58 ± 0.02^{dC}	0.62 ± 0.01^{cC}	0.41 ± 0.01^{fB}	0.52 ± 0.01^{eC}
	20	0.34 ± 0.03^{hA}	0.46 ± 0.03^{gA}	0.68 ± 0.01^{dB}	0.76 ± 0.03^{bA}	0.81 ± 0.01^{aB}	0.65 ± 0.01^{eB}	0.71 ± 0.02^{cB}	0.44 ± 0.01^{gA}	0.54 ± 0.01^{fB}
	40	0.39 ± 0.05^{hA}	0.49 ± 0.01^{fA}	0.75 ± 0.01^{cA}	0.79 ± 0.01^{bA}	0.86 ± 0.03^{aA}	0.69 ± 0.02^{dA}	0.75 ± 0.01^{cA}	0.45 ± 0.01^{gA}	0.59 ± 0.01^{eA}

Rheology evaluation

Viscoelastic assays are valuable and interpretable when perform in the linear range and frequency effect on dynamic modules G′ and G″ is depicted in Fig. 2. The modules variations for all treatments varied from 0.01 to 100 rad/s, but in the range of 0.01 and 10 rad/s, dynamic modules G′ and G″ were enhanced through improving frequency. In these samples, since G' and G" were not parallel to horizontal axis, it could be seen that texture was soft as well as G' was further and AWC_3 had harder tissue compared to others during shelf life. The G′ and G″ for AW_5, AW_6, AC_7, AC_8, AWC_4 and AWC_1 were lower than A owing to NCEO presence (in AC_8, AC_9) and walnut concentration. Therefore, the strongest viscoelastic structure was related to AWC_3 (most protein level) during shelf life, while NCEO alone caused a strong molecular destruction and arrangement between protein and fat; as a result, cheese texture became weak.

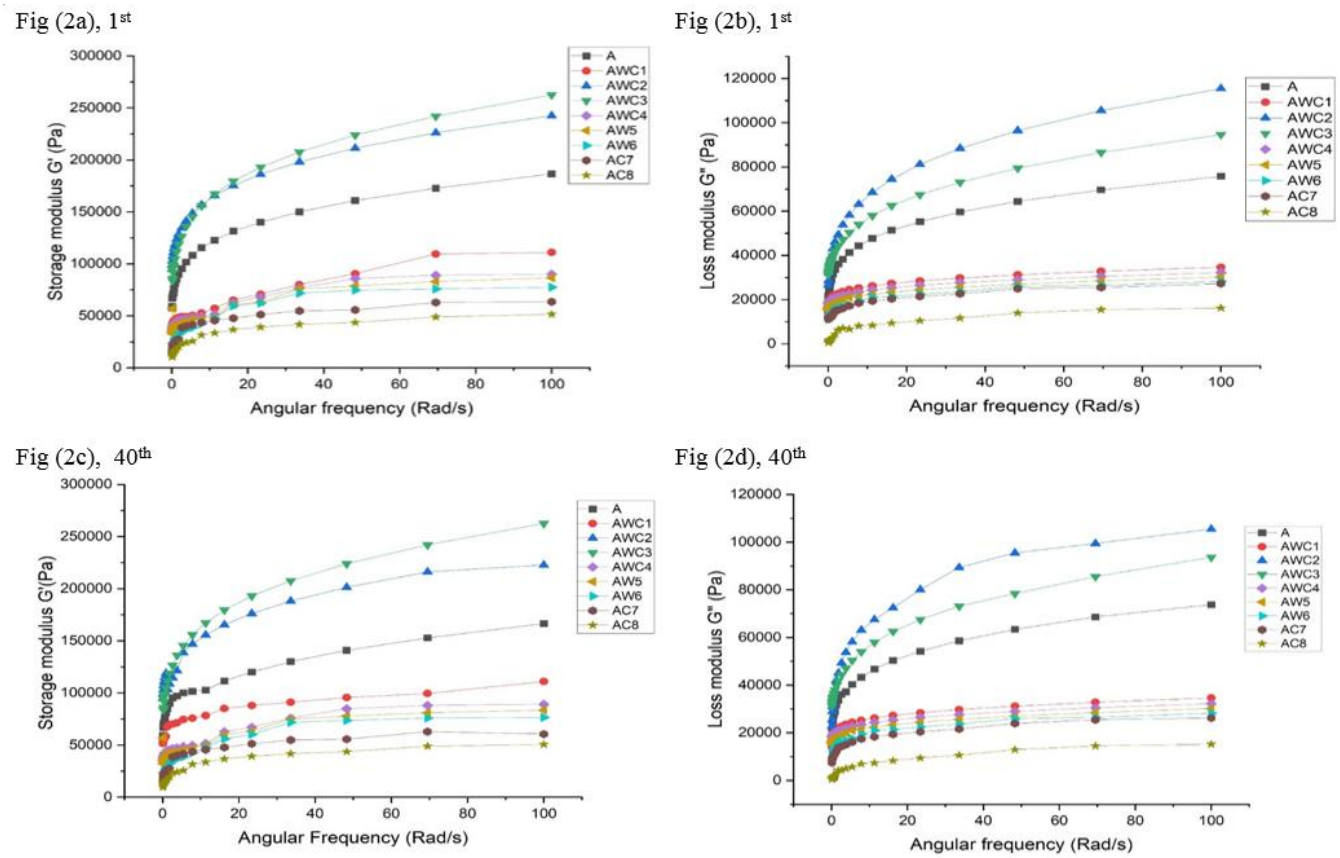


Fig. 2. The effect of frequency on (a) the storage modulus (G′) and (b) loss modulus (G″) in 1st and (c) the storage modulus (G′) and (b) loss modulus (G″) in 40th storage of treatments

Sensory evaluation

The results related to sensory assessment for cheese samples are outlined in Fig. 3; in investigation of color and taste, the highest and lowest points were obtained in AW_6 and AWC_4 during shelf life, so walnut addition had a noticeable influence on these features. AW_6 and AC_8 indicated the maximum and minimum levels for smell, respectively, which illustrated the considerable impact of NCEO and more concentration was not favored. According to texture, the least score was distinguished for A on 20th day and the most was obtained in AWC_3 on 40th day. In overall acceptance, consumers assigned the lowest score to AWC_4 and AC_8 as well as the highest to AWC_3 and AW_6. The more NCEO concentration (0.6 %) was not attractive to consumers and most of them accepted cheese with AWC_3.

Microstructural investigation

The changes in morphological and microstructural attributes of AWC_3 as an optimum sample and A were represented on the 1st and 40th days (Fig. 4). As can be observed in images, AWC_3 had a mesh texture with uniform scattered holes on the 1st day and A indicated a ***homogeneous*** framework and no pores. In this way, fat content of walnut powder and NCEO had been preserved in cheese texture and according to SEM images, AWC_3 exhibited a more compact and coherent structure with cross-links. NCEOs demonstrated great dispersibility in framework; therefore, they could create two phases in many immiscible structures, powerful connections and strengthened matrix owing to their small size and surface activity. In cheese samples on the 40th day, whole size had enhanced due to reduction for moisture and protein as well as increase in fat of AWC_3. Hence, the appropriate use of walnut powder and NCEO could indicated a significant effect on textural and morphological characteristics.

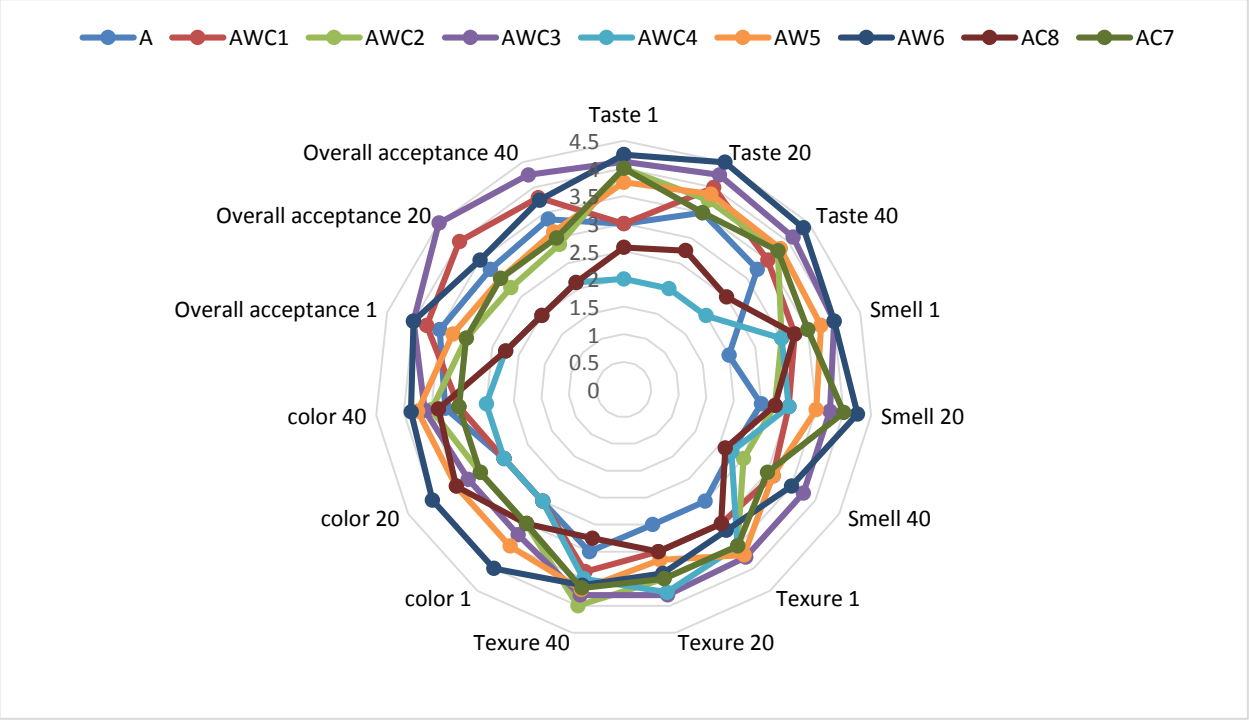


Fig. 3. Sensory evaluation of Aroushe chesses supplemented with different walnut and nanoencapsulated clove essential oil (NCEO) on 1st, 20th, and 40th days of storage.

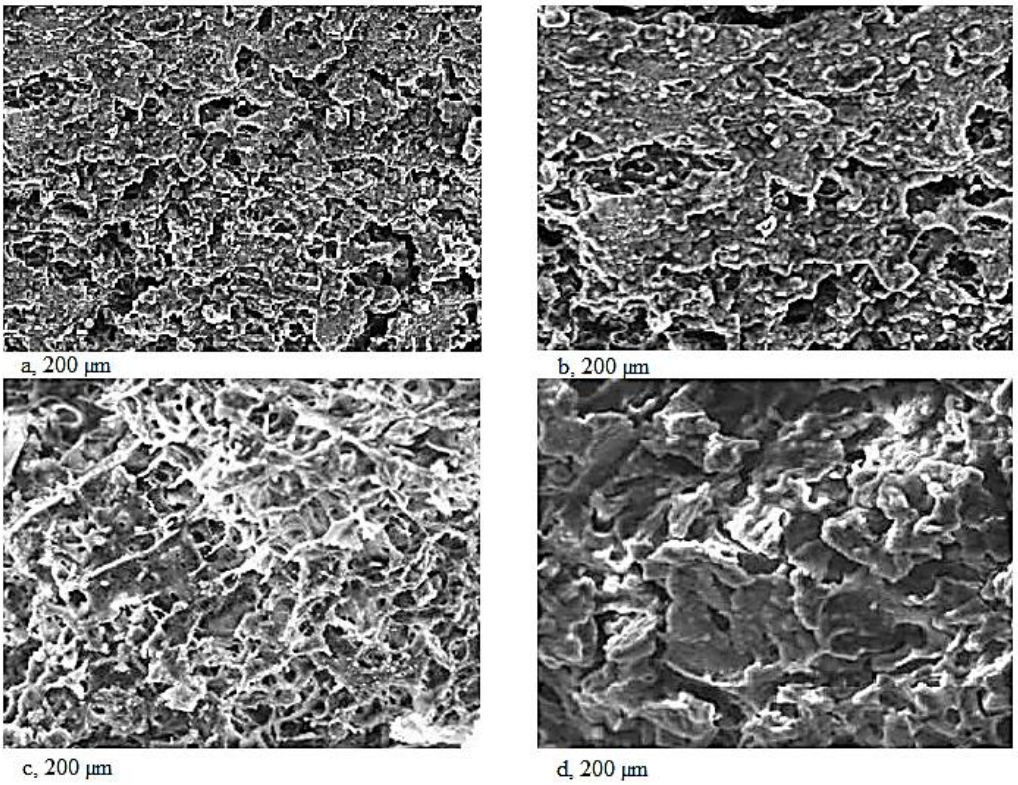


Fig 4. SEM images of (a) A in 1st day, (b) A in 40th day, (c) AWC3 in 1st day, and (d) AWC3 (Aroushe cheese with 3 % walnut and 0.3 % NCEO) in 40th day of storage.

Discussion

PDI percentages for components were extremely further compared to volume and their functions uniquely improved the nanoparticle performance (22).

The CEO release in food product was intensely important for evaluating encapsulation efficiency and adequate applications (14). In present research, the rapid release was observed during 20 days of shelf life; therefore, EO was obtained 70 %. The diffusion molecules for CEO and loaded high oil near nanoparticle surface caused rapid release due to more dissolution of polymer matrix (23). In the next step, CEO release decreased dramatically about 20 % from 20th to 40th days compared to initial rapid rate. Lastly, diffusion was reduced owing to EO concentration gradient between nanoparticles and environment (22). Similar to present results, 80 % release of encapsulated samples had been reported by chitosan nanoparticles during 10 days (9). In another study, 71 % EO release from nanoencapuslated *Ocimum sanctum* was distinguished about 10 h (16).

Fortification with plants in form of EOs and extracts prevented pH enhancement during storage owing to high phenolic components (9). Similar to present results, the application of black cumin in Erzincan Tulum cheese diminished pH compared to control, which was from 5.02 to 4.69 (24). The results illustrated that tomato powder reduced pH for processed cheese, but this feature was elevated after 40 days (25). Moisture was owing to polyphenols caused shrinkage matrix, whey excretion and less trapped water in protein network (21). In evaluation of Erzincan Tulum with black cumin, moisture loss was done by microbial multiplication and acid production that occurred during ripening and syneresis (24). The contents of fat and protein increased slightly in ewe milk cheese with saffron by extending shelf life (26). The investigation of Kashar cheese exhibited that initial protein was 23.9 to 25.3 % for samples during ripening (90 < days) and 26.3 to 29.4 % after specified time (27). The addition of black cumin improved ash content in Erzincan Tulum cheese during the ripening time (24). Furthermore, Kashar cheese (< 90 days of ripening time) indicated that initial ash for samples varied between 3.56 and 4.10 % and also at the end, this level was in the range of 4.44 to 11.6 % (27).

EOs had phytochemical constituents such as phenolic acids, flavonoids and tannins that could be applied as an alternative to synthetic antimicrobials in many products (9). The effect of biological preservatives on microbial functions for cheddar cheese exhibited that coliform was recorded in any sample and also yeast and mold numbers were acceptable during storage (28). It can be observed that *Staphylococcus aureus* was not present during shelf life in fresh cheese with 0.03 % mint EO, which maintained the inhibitory impact (29).

Texture profile analysis measured the response for food products to double-bite deformation and involved key factors of relevance during consumer mastication, simulating several compressions between molar teeth (2). Hardness is defined as the maximum force applied in first bite, which is influenced by agents such as water activity, moisture, protein and fat content (20). The cause for this phenomenon was to be weakness of internal links in structure with more moisture and softer; as a result, cheese shape changed easily and irreversibly against pressure applied by texture measuring device (30). Adhesiveness in cheese was generally further owing to fat enhancement and protein matrix became open and also loose, which led to an improvement in stickiness; while structure was denser and adhesiveness diminished in low fat (2). Cohesiveness demonstrated change in shape that occurred for a sample when was compressed by mill teeth before tearing and it was dependent on intensity of internal bonds, which made up product structure (30). Springiness referred to deformation recovery rate, which a cake piece returned to unreformed state immediately after applied tension was removed (31).

Totally, the addition of walnut and NCEO had improved some textural factors for cheese samples due to protein and fat. The effect of cheese components especially fat, protein and moisture on textural characteristics had been reported in various studies (32, 33). The hardness for artisanal Adobera from Los Altos de Jalisco, a genuine Mexican cheese ranged from 0.86 to 1.93 kg and 0.85 to 1.62 kg; cohesiveness 0.38 to 0.58 and 0.15 to 0.40 as well as springiness 0.52 to 0.77 and 0.38 to 0.58 in dry and rainy seasons, respectively (32). The hardness 10.35 N (1055.41 g), cohesiveness 0.28 N (28.55 g) and gumminess 3.00 N (305.91 g) were represented for Feta cheese (31).

The storage modulus (G') illustrated the energy stored in sample structure, if it was greater than loss modulus (G"), material could be considered mostly elastic and in this assay, it was necessary to determine viscoelastic range (18). G" and G' were constant in linear viscoelastic region and structure changes were negligible and reversible; however, these levels were reduced in non-linear area by increasing strain and irreversible variations (34). This behavior was rheologically known as a weak gel; G′ was higher than G″ in all samples, which confirmed solid viscoelastic properties. When G' < G″ (gel), elastic behavior dominates viscous behavior and for G' > G″ (liquid), this trend is reversed (18). Harder tissue was due to protein and phospholipids with emulsifying agent and also surface activity, it caused a strong interaction between gel components (26). Because oil of sample structure had a lubricating role in the polymer chains, which weakened the matrix and reduced product hardness (31). The dynamic modules of G′ and G″ were declined in all samples on the 40th day, which was probably owing to further fat. The findings of present study illustrated that walnut powder strengthened the gel-like structure and texture in cheese samples; furthermore, more concentration improved G′. Researchers announced that higher fat led to reduced tissue durability because fat droplets had a detrimental influence on protein matrix and weakened emulsion system (35). In line with present finding, fat content diminished from 23 to 6 % in low fat cheese production as well as G′ and G″ modules increased significantly possibly on account of further protein and moisture fractions (18). The literatures displayed that higher fat concentration indicated elevated G″ and lowered G' at less frequencies (25, 36). Some studies stated that replacement of milk fat with vegetable oils had an impact on size and distribution for components in cheese and consequently rheology (26). In general, fat globules were mostly spherical and evenly scattered in protein structure, which was affected when these constituents were modified especially by replacing with vegetable oils (37, 38). Furthermore, G' enhancement was observed for all samples by extending temperature during cooling in similar models of cheese processed by replacing 99 % milk fat with coconut or palm oil (37). The lowest G' was found in products with palm oil and this could be related to fatty acids with a lower melting point specifically oleic acid (37).

In cheese preparation, the taste was not remarkably affected by adding lyophilized extract of arbutus with 0.3 g/L concentration ($p \geq 0.05$) and in greatest level, scores for samples were considerably reduced compared to control (21). Based on evaluation of cheddar cheese enriched with green tea extract, the scores between the samples for smell description did not differ significantly (28). The cheese production and sensory function exhibited that treated samples with an average level of tomato powder (2 %) had the maximum taste scores (25).

The previous researches evaluated tofu microstructure prepared with sesame oil, which found that harder samples had a more uniform structure with adequate cohesion, but in SEM images, large and non-uniform voids were detected in products (2, 18).

Conclusion

Enrichment of edible product with bioactive components has received attention in recent years owing to a pleasant effect on nutritional attributes and acceptability by consumer. Traditional cheese samples are produced in several countries with distinct tastes and consumed abundance almost all over the world. The most and least moisture levels were indicated for control and also AWC_4; then, maximum fat and ash was detected for this treated sample; however, the greatest protein was distinguished in AWC_3. No microbial growth was observed for all treatments on the 1st and 20th days of storage; hence, the minimum and maximum hardness was represented for A

and AWC_3 according to texture analysis, respectively. Generally, walnut powder and NCEO had improved some texture factors for cheese samples on account of protein and fat. The walnut application strengthened the gel like structure in cheeses and adding concentration developed G′, which illustrated an increase for texture hardness. Regarding to overall acceptability in sensory evaluation and appropriate results, AWC_3 was the most interesting treated cheese and also had necessary potential for industrial production and available to public.

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