# Impact of Using Freeze-Shocked Adjunct Cultures on Edam Cheese Quality During Ripening

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***Received:*** *16/4/2023*

**Abstract:** Aiming to enhance the proteolysis of Edam cheese through using 1% freeze-shocked *Lactobacillus* (freezed for 96 hr/-18°C) on cheese curd based on the quantity of cheese-milk and followed its impact on the quality characteristics. Edam cheese was made with traditional starter to serve as control cheese. While other treatments were manufactured by using traditional starter, plus 1% freeze shocked of *Lactobacillus casei* ($T_1$), *Lb. helveticus* ($T_2$) or *Lb. plantarum* ($T_3$). The changes in the composition, ripening indices, rheological properties, and sensory attributes of Edam cheese from different treatments were followed during ripening period (60 days). Freeze- shocked treated cheeses had no significant effect on the gross composition with lower pH values as compared to control cheese. Also using freeze-shocked lactobacilli in Edam cheese enhanced all the ripening indices expressed as soluble nitrogen coefficient, non-protein nitrogen coefficient, free amino acids, and total volatile fatty acids. The rheological characteristics of Edam cheese with freeze-shocked cultures had lower hardness, cohesiveness, gumminess, and chewiness than control. Edam cheese made with *Lb. helveticus* ($T_2$), showed the highest proteolysis rate, while showing significantly lower hardness, gumminess, and improved sensory attributes than control cheese. Using freeze-shocked *Lb. helveticus*, *Lb. casei* and *Lb. plantarum* as adjunct culture for Edam cheese making had a pronounced effect on both flavor and body and texture of the resultant cheeses and reduced the ripening period to 45 days instead of 60 days for control cheese. Using 1% *Lb. helveticus* as adjunct culture on cheese curd improved the cheese quality more effectively.

***Keywords:*** Edam cheese, Freeze-shocked lactobacilli, Rheological characteristics, Sensory properties

## INTRODUCTION

Edam cheese, a semi-hard Dutch cheese variety, contains about 40 – 44 % fat content in solid matter and ripened for two weeks to around two years. This type is manufactured in the form of sphere loaf weighing about 0.9 - 2 kg. The smooth-textured interior of Edam is a rich yellow-gold, with a mild but savoury lavour that makes it popular as a snack cheese. The quality of cheese characteristics is highly affected by the quality of cheese-milk with a good bacteriological quality and a standardized chemical composition (Fox et al., 2017). Different groups of microorganisms can be present in cheese generally through the surrounding environment or post-contamination after pasteurization. Some of these microorganisms had a role in the development of flavour, body texture, and some of them are undesirable which cause some defects in this cheese. Egyptian Standards (2005) reported the chemical and microbial standards of Edam cheese as follows: dry matter % and Fat on dry matter (F/D.M) must be not less than 54 and 40%; respectively, as well the final product must be free from pathogenic microorganisms, Coliforms, yeasts, and molds must be not exceeding 10,100 and 10 cfu/g of cheese respectively.

Cheese ripening is a complex biochemical process by which the rubbery curd is transformed into a smooth body and texture and fully flavoured cheese. Both of flavour and body& texture are considered as the two main criteria in cheese acceptability of aged cheese. The time that is required to develop the original flavour and texture varies from a few weeks for soft cheeses up to a few years for very hard varieties. During this period, cheeses attain their own characteristics through series of chemical, microbiological and biochemical changes whereby fat, protein and residual lactose are broken down to primary products which are further degraded to secondary products which participate more extensively in cheese flavour (McSweeney and Fox, 1997; Kheadr et al., 2003).

Acceleration of cheese ripening have been paid more attention for several scientists and stakeholder`s to offer a cheese with rich organoleptic properties in shorter ripening period, so scientists focused their efforts towards enhancing cheese ripening. Several strategies were proposed to accelerate cheese ripening such as use of exogenous enzymes and encapsulated enzymes, genetically engineered starters, cheese slurries, adjunct and attenuated adjunct cultures, high temperature and pressure and concentrated source of substrates as enzyme-modified cheese powder (Azarnia et al., 2011; Khattab et al., 2019)

Adjunct bacterial cultures and non-starter lactic acid bacteria (NSLAB) are the principal contributors to the ripening process by which most of the cheese flavour develops. The variety in cheese flavour and aroma across the different types arises from the incorporation of different secondary flora in the production process (Andiç et al., 2014) *Lactobacillus helveticus* and *Lactobacillus*

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*casei* are bacterial strains with high activities of aminopeptidase, dipeptitase and protease enzymes, besides, they retain in the cheese matrix (Pettersson and Sjöström 1975). For this reason, lactobacilli cells modified by the physical methods like freeze-shocked have been used to accelerate the ripening in varieties of many cheeses such as Cheddar (El-Soda et al. 1991), Swedish hard cheese (Ardö and Pettersson 1988), Gouda (El-Nagar et al. 2010) and Ras cheese (Kebary et al., 1996). *Lactobacillus plantarum* strains are widely used as adjunct cultures for accelerated ripening during production of different types of cheeses (Gomez et al., 1996, Spus et al., 2017). Because *Lb. plantarum* strains possess a potent collection of enzymes, including cell envelope–bound proteinases and intracellular peptidases, such enzymes, when released into the cheese matrix, may influence proteolysis, as previously shown in Cheddar cheese (Griffiths and Tellez, 2013). Freeze-shocked lactobacilli cultures can enhance flavour and accelerate different cheese varieties during ripening because of induced autolysis without increasing the cheese acidities. Since it hydrolyze more casein and release various bioactive compounds (Kebary et al. 1996 (Ras cheese); Madkor et al., 2000 (Cheddar cheese); Gürsoy, 2009 (Kaşar cheese); El-Tanboly et al., 2010 (Gouda cheese); El-Ataar, 2015 (Kachcaval cheese); Calasso et al., 2020 (Caciotta cheese). Therefore, the objective of this study was to evaluate the impact of using freeze shocked lactobacilli species on the quality characteristics of Edam cheese during ripening.

## MATERIALS AND METHODS

Fresh cow's milk (11.88% dry matter, 3.0 % fat, 2.4% casein, casein/ fat ratio 0.8 and pH value 6.65) was obtained from the dairy processing unit, dairy department, Faculty of Agriculture, Suez Canal University, Ismailia governorate, Egypt. Skim milk powder (T.S 97%) (Grade A-low heat-spray process-pasteurized) - USA was obtained from the local market. The starter cultures (FD-DVS CHN-11, mesophilic aromatic culture) consist of *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Leuconostoc mesenteroides* subsp. *mesenteroides, Leuconostoc mesenteroides* subsp. *cremoris*) and *Lactococcus lactis* subsp. *lactis biovar diacetilactis*). This culture was obtained from CHR-Hansen's laboratories, Denmark by MIFAD Company for food additives, Cairo, Egypt. Pure strains of *Lactobacillus helveticus* DSMZ 20082 (*Lb. helveticus*), *Lactobacillus casei* (*Lb. casei* 101) and *Lactobacillus plantarium* LPU4 (*Lb. plantarium*) were obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbial Resources Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt. Rennet powder (CHY-MAX, 2280 IMCU/ml) was obtained from CHR-Hansen's laboratories, Denmark. Commercial pure fine grade salt (NaCl) was obtained from Emisal Company, Egypt. Calcium chloride (Food quality grade) was obtained from EL-Nasr Company, Cairo, Egypt. All chemicals and reagents used in this study were analytical grade.

**Propagation of different cultures and its Freeze-shocked cells:**

The culture was sub-cultured for 12 hrs at 37ºC in 11.5% reconstituted skim milk powder at least twice before using. The different cultures of Lactobacilli including *Lb. helveticus, Lb. Casei* 101 and *Lb. Plantarium* were freeze-shocked as described by Bartels et al., (1987). Total bacterial counts (TBC) were determined using plate count agar according to Houghtby et al., (1992) before and after freezing.

***Manufacture of Edam cheese:***

Edam cheese was manufactured according to the method described by Scott (1981) with using the traditional starter. The cheese curd was divided into 4 portions. The first was used as a control for comparison. Other three cheese curd portions were mixed individually with freeze-shocked cultures of *Lb. casei, Lb. helveticus* and *Lb. plantarum* at 1% calculated as cheese-milk quantity on cheese curd respectively to prepare T1, T2 and T3 respectively.

***Analysis of cheese milk:***

Total solids, fat, (casein content of milk by formol titration) and titratable acidity of cheese milk were determined according to AOAC (2007). Values of pH were measured using Jenway 3505 pH meter, Jenway limited, Gransmore green, Felsted, Dunmow, England.

***Analysis of cheese:***

Moisture contents by drying method, fat content using Gerber method, total protein, total nitrogen content by Kjeldahl method using semi-micro Kjeldahl and total acidity by titration method were determined according to the method described in AOAC (2007). Water soluble nitrogen (WSN) and non-protein nitrogen (NPN) contents using trichloroacetic acid 12% were prepared and determined according to the method described in AOAC (2007). Salt was measured according to Volhard method as described by AOAC (2007). The values of pH were measured using Jenway pH meter with Jenway spear electrode No: 3505. Free amino acids value was estimating using cadmium-ninhydrin method as described by Folkertsma and Fox (1992). Total volatile fatty acids (TVFA) contents were estimated by the distillation method according to Koiskowski (1982).

***Texture profile analysis (TPA):***

Texture profile analyses (TPA) of experimental Edam cheeses were carried out by a Universal Testing Machine, TMS-pro (Stable Microsystems, Godalming, UK) according to method described by Gunasekaran and Ak, (2002). Formulations, in the original containers, were compressed at a depth of 8 mm using a type P 25/L acrylic cylinder probe with compression rate of 2 mm/s and force of 0.10 N for 5 s. The output of the machine is a table contains some cheese rheological characteristics such as hardness, springiness and cohesiveness. Both of Gumminess and Chewiness can be calculated from the obtained results as follows: Gumminess = Hardness × Cohesiveness, Chewiness = Gumminess × Springiness.

***Organoleptic properties:***

The organoleptic properties of cheese samples were examined as described by Clark et al. (2009) with maximum score points 50, 40 and 10 for flavour, body& texture and appearance respectively. The organoleptic properties were evaluated after 15, 30, 45 and 60 days of ripening by trained staff members of Dairy Department, Faculty of Agriculture, Suez Canal University.

**Statistical analysis:**

All measurements were done in triplicate, and analysis of variance with two factorial (treatments and period. It was found that the rate of decreasing in moisture content was higher in the first month with descending rates through ripening period for all cheese treatments. Similar results were reported by El-Tanboly et al. (2010) and El-Nagar et al. (2010) for Gouda cheese treated by freeze-shocked cultures. The moisture content of Edam cheese during ripening periods were significantly ($p \leq 0.01$) different. This may be due to the surface loss of moisture during ripening. Similar finding was reported by Hamdy et al. (2022). This decreases in moisture contents caused gradual increases for all constituents of cheese during ripening. All cheese treatments are conformed to the Egyptian Standards (2005). All the aged cheeses contained moisture and fat on dry matter contents within the reported limits in Edam cheese (Nalepa et al., 2020; Abdelmontaleb et al., 2020; Egyptian Standard of Edam cheese, 2005). Salt on moisture phase had no significant effect between treatments but during ripening period, had a significant effect as a result of higher salt content during ripening with decreasing trend for moisture contents for all treatments. Similar finding was reported by El-Aidie et-al. (2019). Generally, as the ripening period of Edam cheese advanced, pH value of all cheeses tended to decrease significantly ($p \leq 0.01$) as a result of production of acidic compounds through fermentation of residual lactose and degradation of intermediates components of protein and fat (El-Kholy, 2015). Similar findings were reported by Abdelmontaleb et al. (2020). Treated Edam cheeses (Table 2) had significantly ($p \leq 0.01$) lower pH values than control when fresh or throughout the ripening process. So, using freeze-shocked lactobacilli in Edam cheese caused gradual decreases with different rates than control as affected by the excessive viable cells of freeze- shocked cultures to utilize lactose and produce more lactic acid caused parallel decreases for the pH values. So, the differences among treatments are significant ($p \leq 0.01$). The lowest pH value of treated cheeses throughout the ripening period was that treated with *Lb. plantarum* followed by *Lb. casei* and lastly that treated by *Lb. helveticus*. Similar results were reported by El-Deeb et al. (2020) for Gouda cheese treated by adjunct *Lb. helveticus* and *Lb. plantarum*. These results are in agreement with the ripening period) were conducted by the procedure of General Linear Model (GLM) according to Snedcor and Cuchran (1967) using Costat under windows software version 6.311 and least significant difference (LSD) test were employed to determine significant difference at $p \leq 0.01$.

## RESULTS AND DISCUSSION

**The impact of freeze-shocked on lactobacilli counts and its activities:**

The activities of different Lactobacilli strains after preparation and after freeze storage for 96 hours at -18ºC are illustrated in Table (1). It appeared that the higher total count of different lactobacilli strains was found in freshly prepared. The highest total count among these strains was *Lb. plantarum* followed by *Lb. casei* and the least was *Lb. helveticus*. Freeze-shocked lactobacilli for 96 hours at -18 ºC caused decreases in its total count of these strains as showed in Table (1). The reduction in total count of these strains by freezing may be due to the damage occurred in cell wall and membrane resulting cell lysis with partially decrease its ability to ferment lactose (Thiboutot et al., 1995 and Kandil and El Soda, 2015). But generally, the rate of decreases for the tested cultures was limited as a result of freezing is commonly used for the preservation and storage of starter cultures used in the dairy industry.

**Table (1): The total count (T.C.) of different Lactobacilli strains when prepared and after freeze storage (-18 ºC/96 hours)**

| Lactobacilli strains | Zero time | After 96 hours freeze-shocked |
|---|---|---|
| T.C. of *Lb. casei* | $18\times10^6$ | $10\times10^6$ |
| T.C. of *Lb. helveticus* | $32\times10^5$ | $13\times10^5$ |
| T.C. of *Lb. plantarum* | $38\times10^6$ | $17\times10^6$ |

**Gross chemical composition:**

The gross chemical composition of different Edam cheese treatments as affected by using freeze-shocked lactobacilli is seen in Table (2). Generally, treated cheeses with freeze-shocked lactobacilli had insignificant ($p \leq 0.01$) effect on moisture, total protein, salt contents and fat on dry matter values higher moisture content than control throughout the storage.

findings of Gomez et al. (1996) who found that semi-hard cheese treated with *Lb. plantarum* had significantly lower pH than that treated with *Lb. helveticus*. At the end of ripening period, pH of treated cheeses raised as a result of the further decomposition of lactate, the decarboxylation of the free amino acids, the oxidation of fatty acids and for the liberation of ammonia (McSweeney, 2004)

**Table (2): Changes of gross composition and pH value of Edam cheese as affected by using freeze-shocked Lactobacilli during ripening periods (average of three replicates)**

| parameters | Treatments | Ripening Periods (days) | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | | Fresh | 15 | 30 | 45 | 60 | |
| Moisture % | C | 45.28 | 44.03 | 43.17 | 42.69 | 42.44 | 43.51 A |
| | $T_1$ | 45.05 | 43.83 | 42.94 | 42.30 | 42.01 | 43.23 A |
| | $T_2$ | 45.13 | 44.01 | 43.13 | 42.55 | 42.16 | 43.30 A |
| | $T_3$ | 44.97 | 43.70 | 42.80 | 42.25 | 41.85 | 43.12 A |
| | Mean | 45.11 A | 43.89 B | 43.01 C | 42.45 D | 42.12 E | |
| Fat on Dry matter (F/DM) | C | 40.83 | 41.32 | 41.64 | 42.02 | 42.27 | 41.60 A |
| | $T_1$ | 40.80 | 41.39 | 41.62 | 42.03 | 42.39 | 41.65 A |
| | $T_2$ | 40.70 | 41.09 | 41.47 | 41.78 | 42.22 | 41.51 A |
| | $T_3$ | 40.74 | 41.15 | 41.52 | 41.85 | 42.28 | 41.46 A |
| | Mean | 40.76 E | 41.24 D | 41.57 C | 41.92 B | 42.29 A | |
| Total Protein (TP) | C | 23.55 | 23.73 | 24.21 | 24.54 | 24.75 | 24.12 A |
| | $T_1$ | 23.29 | 23.93 | 24.44 | 24.82 | 25.14 | 24.44 A |
| | $T_2$ | 23.35 | 24.05 | 24.56 | 25.01 | 25.39 | 24.31 A |
| | $T_3$ | 23.41 | 24.10 | 24.69 | 25.15 | 25.46 | 24.56 A |
| | Mean | 23.40 E | 23.95 D | 24.47 C | 24.88 B | 25.19 A | |
| Salt % | C | 1.41 | 1.53 | 1.61 | 1.67 | 1.72 | 1.59 A |
| | $T_1$ | 1.45 | 1.51 | 1.58 | 1.65 | 1.70 | 1.58 A |
| | $T_2$ | 1.41 | 1.50 | 1.58 | 1.64 | 1.71 | 1.57 A |
| | $T_3$ | 1.43 | 1.49 | 1.57 | 1.66 | 1.71 | 1.56 A |
| | Mean | 1.42 E | 1.51 D | 1.58 C | 1.65 B | 1.71 A | |
| Salt on Moisture phase (SMP) | C | 3.18 | 3.47 | 3.73 | 3.91 | 4.05 | 3.67 A |
| | $T_1$ | 3.22 | 3.45 | 3.68 | 3.90 | 4.05 | 3.66 A |
| | $T_2$ | 3.12 | 3.39 | 3.66 | 3.85 | 4.04 | 3.65 A |
| | $T_3$ | 3.17 | 3.41 | 3.67 | 3.93 | 4.09 | 3.64 A |
| | Mean | 3.18 E | 3.43 D | 3.68 C | 3.90 B | 4.06 A | |
| pH value | C | 5.72 | 5.56 | 5.47 | 5.41 | 5.35 | 5.50 A |
| | $T_1$ | 5.68 | 5.50 | 5.40 | 5.33 | 5.37 | 5..46 C |
| | $T_2$ | 5.70 | 5.51 | 5.43 | 5.39 | 5.42 | 5.48 B |
| | $T_3$ | 5.67 | 5.48 | 5.37 | 5.30 | 5.33 | 5.43 D |
| | Mean | 5.69 A | 5.51 B | 5.42 C | 5.69 D | 5.31 E | |

* Means with the same letter among treatments or during ripening periods are not significantly different ($p \leq 0.01$).
C: control cheese made with traditional starter
T1: Edam cheese made with traditional starter with *Lb. casei* as adjunct culture.
T2: Edam cheese made with traditional starter with *Lb. helveticus* as adjunct culture.
T3: Edam cheese made with traditional starter with *Lb. plantarum* as adjunct culture.

**Ripening indices of Edam cheese:**

The differences in ripening indices of Edam cheese as affected by using freeze-shocked *Lb. casei., Lb. helveticus* and *Lb. plantarum* during ripening period are illustrated in Table (3). Treated cheeses with freeze-shocked lactobacilli had significantly ($p \leq 0.01$) higher ripening indices expressed as soluble nitrogen (SN), soluble nitrogen coefficient (SN/TN), non-protein nitrogen (NPN) contents, non-protein nitrogen coefficient (NPN/TN), total free amino acids (FAA) and total volatile fatty acids (TVFA) than control cheese when fresh or throughout the ripening period. Generally, the release of SN in cheese is primarily a result of casein breakdown by proteolytic enzymes. As ripening advancing, soluble nitrogen of all cheese treatments was increased significantly ($p \leq 0.01$) as a result of microbial and enzymatic activities which leading to protein decomposition and fat hydrolysis (Law, 1997). Similar findings were reported by El-Desoki and Nasr (2014), El-Aidie et al. (2019) and Hamdy et al.(2022)

Bartels et al. (1987) reported that Edam cheese treated with 1% freeze-shocked lactobacilli tended to have higher ($p \leq 0.01$) levels of SN/TN, NPN/TN and FAA than control because of the lyses of freeze-shocked cells with greater rates than the normal cells.

**Table (3): Changes of ripening indices of Edam cheese as affected by using freeze-shocked Lactobacilli during ripening periods (average of three replicates).**

| Parameters | Treatments | Ripening Periods (days) | | | | | Mean |
|---|---|---|---|---|---|---|---|
| | | Fresh | 15 | 30 | 45 | 60 | |
| Soluble Nitrogen (SN) | C | 0.201 | 0.303 | 0.419 | 0.514 | 0.619 | 0.411 D |
| | $T_1$ | 0.207 | 0.364 | 0.501 | 0.626 | 0.771 | 0.490 B |
| | $T_2$ | 0.214 | 0.391 | 0.548 | 0.702 | 0.822 | 0.533 A |
| | $T_3$ | 0.204 | 0.359 | 0.495 | 0.612 | 0.745 | 0.476 C |
| | Mean | 0.207 E | 0.354 D | 0.491 C | 0.608 B | 0.728 A | |
| Soluble Nitrogen Coefficient (SN/TN) | C | 5.44 | 8.14 | 11.05 | 13.37 | 15.95 | 10.79 D |
| | $T_1$ | 5.67 | 9.71 | 13.08 | 16.08 | 19.06 | 12.72 B |
| | $T_2$ | 5.86 | 10.37 | 14.32 | 17.61 | 20.65 | 13.74 A |
| | $T_3$ | 5.56 | 9.51 | 12.79 | 15.25 | 18.05 | 12.22 C |
| | Mean | 5.63 E | 9.43 D | 12.79 C | 15.58 B | 18.43 A | |
| Non-Protein Nitrogen (NPN) | C | 0.079 | 0.149 | 0.232 | 0.302 | 0.381 | 0.229 D |
| | $T_1$ | 0.086 | 0.195 | 0.315 | 0.421 | 0.529 | 0.309 B |
| | $T_2$ | 0.089 | 0.209 | 0.368 | 0.452 | 0.572 | 0.338 A |
| | $T_3$ | 0.084 | 0.198 | 0.308 | 0.412 | 0.492 | 0.299 C |
| | Mean | 0.084 E | 0.188 D | 0.306 C | 0.397 B | 0.493 A | |
| Non-protein Nitrogen coefficient (NPN/TN) | C | 2.14 | 4.01 | 6.11 | 7.85 | 9.82 | 5.99 D |
| | $T_1$ | 2.35 | 5.20 | 8.22 | 10.83 | 13.43 | 8.01 B |
| | $T_2$ | 2.43 | 5.54 | 9.56 | 11.53 | 14.37 | 8.68 A |
| | $T_3$ | 2.28 | 5.24 | 7.96 | 10.45 | 12.32 | 7.65 C |
| | Mean | 2.30 E | 4.99 D | 7.96 C | 10.17 B | 12.48 A | |
| Free Amino Acids value (FAA)*** | C | 0.011 | 0.094 | 0.211 | 0.256 | 0.302 | 0.175 D |
| | $T_1$ | 0.011 | 0.117 | 0.258 | 0.348 | 0.409 | 0.237 B |
| | $T_2$ | 0.012 | 0.126 | 0.274 | 0.387 | 0.464 | 0.257 A |
| | $T_3$ | 0.011 | 0.111 | 0.235 | 0.32 | 0.367 | 0.210 C |
| | Mean | 0.011 E | 0.121 D | 0.245 C | 0.328 B | 0.386 A | |
| Total Volatile Fatty Acids (TVFA)**** | C | 1.05 | 1.45 | 1.98 | 2.69 | 3.11 | 2.06 D |
| | $T_1$ | 1.08 | 1.61 | 2.45 | 3.28 | 4.10 | 2.5 B |
| | $T_2$ | 1.10 | 1.67 | 2.61 | 3.51 | 4.44 | 2.74 A |
| | $T_3$ | 1.07 | 1.55 | 2.31 | 3.08 | 3.80 | 2.44 C |
| | Mean | 1.07 E | 1.66 D | 2.41 C | 3.14 B | 3.92 A | |

* Means with the same letter among treatments or during ripening periods are not significantly different ($p \leq 0.01$).
** C, $T_1$, $T_2$ and $T_3$: see Table (2).
*** FAA expressed as absorbance at 507 nm / 100 µl of soluble nitrogen of cheese.
**** TVFA expressed as ml of NaoH 0.1 N/ 10 gm of cheese.

The lyses of more bacterial cells will cause a greater release of intracellular enzymes: amino peptidases, dipeptidases and other peptide hydrolyzing enzymes (El-Soda et al., 1993) which accelerate the rate of proteolysis during cheese ripening. The highest ripening indices expressed as soluble nitrogen coefficient, non-protein nitrogen coefficient and FAA among treatments throughout the ripening period was reported for Edam cheese treated by Lb. helveticus followed by Lb. casei followed by treated by Lb. plantarum and at least the control cheese. Madkor et al. (2000) found that Cheddar cheese treated with Lb. helveticus had significantly higher soluble nitrogen coefficient and non-protein nitrogen coefficient than these made with Lb. casei throughout the ripening periods . Using freeze-shocked lactobacilli in Edam cheese increased significantly ($p \leq 0.01$) TVFA throughout the ripening period as compared to Control. The increase of TVFA during ripening period could be due to the residual activity of heat resistance lipase which may cause the fat hydrolysis (Mansour, 2005). Also, the higher levels of free amino acids can be served as precursors for fatty acids (Khalil et al., 2010). The highest ($p \leq 0.01$) rate of lipolysis expressed as TVFA among treatments during ripening was that treated with *Lb. helveticus* followed by *Lb. casei* and *Lb. plantarum*. Similar findings were reported by Tungjaroenchai et al. (2004) and Zaki and Salem (1992) for Edam cheese made with lactobacilli as adjunct culture. Also, El-Deeb et al. (2020) found that Gouda cheese made with Lb. helveticus had higher TVFA than that made with *Lb. plantarum*. Madkor et al. (2000) reported that Cheddar cheese made with Lb. helveticus had higher free fatty acids than that made with Lb. casei.

**The Rheological Characteristics:**

The rheological characteristics of Edam cheese as affected by using freeze-shocked lactobacilli were seen in Table (4). Hardness values can be defined the peak force recorded at 60% compression. From the obtained data, the hardness values of different cheese treatments increased in the early ripening period. This may be due to the decreasing of moisture content. Tunick et al. (1991) reported that decreasing the moisture content in cheese results in a firmer texture due to the alteration in the casein matrix, therefore, increase the hardness. At the end of ripening period, these values decreased because of the enzymatic hydrolysis of the caseins in particular, αs1-casein and peptides, some of which are water-soluble and so cannot contribute to the protein matrix responsible for cheese rigidity (Lane et al., 1997). So, treated Edam cheeses had significantly ($p \leq 0.01$) lower hardness values than control. While the cohesiveness values of different treatments decreased gradually with different rate; the rate of decrease in the early period was slower than the late period as a result of the continuous breakdown of casein matrix with a slight moisture loss at the end of ripening period. Similar finding was reported by El-Deeb et al. (2020) for Gouda cheese. The obtained results revealed that Edam cheese made with using freeze-shocked lactobacilli ($T_1$, $T_2$ and $T_3$) at late ripening periods were significantly ($p \leq 0.01$) lower for both the hardness and cohesiveness values than the control because of its higher proteolysis rates than control. Adda et al. (1982) reported that the hydrolysis of casein during cheese ripening produces compounds that are very soluble in water and do not contribute to the protein network. For this reason, the cheese softens during maturation. Similar findings were reported by Khalil et al. (2010) and El-Deeb et al. (2020) for Trappist and Gouda cheeses made with adjunct lactobacilli.

The maximum values of springiness are present in fresh cheeses. These differences in the springiness during ripening may be attributed to the continuous breakdown of protein matrix and its strength, the latter being dependent on some factors such as moisture, protein and fat content of the cheese (Lawrence et al., 1983). El-Deeb et al. (2020) also reported a decrease in springiness of Gouda cheese with increasing the proteolysis during ripening. The same trend was observed for Edam and Trappist cheeses made from cow's milk by El-Tawel (2004) and Khalil et al. (2010). Treated Edam cheeses with different freeze-shocked lactobacilli had significantly ($p \leq 0.01$) lower values of springiness values than Control as a result of higher proteolysis rates of these treated cheeses as compared to Control Cheese.

Gumminess of cheese is the product of multiplication of cheese hardness and cohesiveness. While chewiness of cheese referred to the product of multiplication of cheese gumminess and springiness. Generally, the gumminess and chewiness increased significantly ($p \leq 0.01$) up to 30 days of ripening as a result of higher losses of moisture contents. Then, these values decreased significantly ($p \leq 0.01$) till the end of ripening period as a result of the continuous breakdown of cheese matrix during ripening. It was found that the gumminess and chewiness values of treated cheeses with freeze-shocked lactobacilli especially during the late ripening periods were significantly ($p \leq 0.01$) lower than that of control. This result is agreement with the obtained results for hardness and cohesiveness of these treatments. Similar trend was reported by Khalil et al. (2010) and El-Deeb et al. (2020) for Trappist and Gouda cheeses treated with adjunct lactobacilli in the same order.

**The Organoleptic properties:**

The organoleptic properties of Edam cheese as affected by using freeze-shocked lactobacilli cultures during the ripening period are presented in Table (5). During the first 15 days of ripening, Control cheese had a firm consistency with a flat flavour and gained total acceptability score 73 points out of 100. After 30 days of ripening, it was characterized for flavour as mild and for smooth body& texture with few eyes and it possessed a total score of 77.5 points out of 100. After 45 days of ripening, the same treatment was characterized for flavour as typical flarour cheesy and for body & texture were smooth with few eyes and ranked a total score 82 out of 100. At the end of ripening period, the cheeses were characterized as full flavour ripened and smooth body& texture with few eyes and gained total scores 87 out of 100 points. for Gouda cheese, Kebary (1996) for Ras cheese and khalil et al. (2010) for Trappist cheese. They reported that treated cheeses supplemented with freeze-shocked *Lb. helveticus* had higher flavour intensity.

Edam cheese made with *Lb. plantarum* ($T_3$) enhanced the organoleptic properties of the resultant cheese and gained significantly ($p \leq 0.01$) higher total acceptability scores than Control. But these scores are significantly lower than $T_1$ and $T_2$ when fresh and during the ripening period. After 30 days of ripening, the cheese was characterized for flavour as slightly flavour and smooth body with few eyes and scored 78.5. While at the end of ripening period, the cheese is characterized for flavour as cheesy flavour and scored a total score 88.5 out of 100 points. This may be correlated to the lowest ripening indices of this treatment among treated cheeses. Similar results were reported for Gouda cheese made with *Lb. helveticus* and *Lb. plantarum* (El wahsh et al., 2020) who found that total acceptability scores of Gouda cheese made with *Lb. helveticus* had higher total acceptability scores than that made with *Lb. plantarum*. Similar trend was found for Cheddar cheese made with freeze shocked *Lb. helveticus* which gained higher body& texture and flavour scores than that made with *Lb. casei* (Madkor et al., 2000).

**Table (4): Changes of rheological characteristics of Edam cheese as affected by using freeze-shocked Lactobacilli during ripening periods (average of three replicates)**

| Parameters | Treatments | Ripening Periods (days) | | | Mean |
|---|---|---|---|---|---|
| | | Fresh | 30 | 60 | |
| Hardness (N) | C | 9.85 | 14.52 | 11.32 | 11.94 A |
| | $T_1$ | 9.92 | 14.35 | 11.22 | 11.87 C |
| | $T_2$ | 9.88 | 14.40 | 11.12 | 11.81 D |
| | $T_3$ | 9.94 | 14.48 | 11.24 | 11.91 B |
| | Mean | 9.90 C | 14.46 A | 11.23 B | |
| Cohesiveness | C | 0.75 | 0.68 | 0.58 | 0.68 A |
| | $T_1$ | 0.76 | 0.67 | 0.54 | 0.65 C |
| | $T_2$ | 0.76 | 0.66 | 0.52 | 0.64 D |
| | $T_3$ | 0.77 | 0.68 | 0.55 | 0.66 B |
| | Mean | 0.76 A | 0.67 B | 0.55 C | |
| Springiness (mm) | C | 6.70 | 5.72 | 4.85 | 5.76 A |
| | $T_1$ | 6.48 | 5.38 | 4.40 | 5.42 C |
| | $T_2$ | 6.40 | 5.32 | 4.28 | 5.33 D |
| | $T_3$ | 6.50 | 5.46 | 4.42 | 4.46 B |
| | Mean | 6.52 A | 5.47 B | 4.48 C | |
| Gumminess (N) | C | 7.39 | 9.87 | 6.57 | 7.95 A |
| | $T_1$ | 7.54 | 9.61 | 6.06 | 7.74 C |
| | $T_2$ | 7.51 | 9.50 | 5.78 | 7.59 D |
| | $T_3$ | 7.67 | 9.85 | 6.18 | 7.89 B |
| | Mean | 7.53 B | 9.70 A | 6.15 C | |
| Chewiness (N.mm) | C | 49.50 | 56.48 | 31.84 | 45.94 A |
| | $T_1$ | 48.85 | 51.73 | 26.66 | 42.41 C |
| | $T_2$ | 48.06 | 50.56 | 24.75 | 33.79 D |
| | $T_3$ | 49.85 | 53.76 | 27.32 | 43.64 B |
| | Mean | 49.06 C | 53.13 A | 27.64 C | |

* Means with the same letter among treatments or during ripening periods are not significantly different ($p \leq 0.01$).

** C, $T_1$, $T_2$ and $T_3$: see Table (2).

**Table (5): Changes of organoleptic properties of Edam cheese as affected by using freeze-shocked Lactobacilli during ripening periods (average of three replicates)**

| Treatments | Ripening periods (days) | Falvour (50 points) | Body& Texture (40 points) | Appearance (10 points) | Total acceptability (100 points) |
|---|---|---|---|---|---|
| C | 15 | 33 | 33 | 7 | 73C |
| | 30 | 35 | 34.5 | 8 | 77.5D |
| | 45 | 37.5 | 36.5 | 8 | 82D |
| | 60 | 40 | 38 | 9 | 87D |
| $T_1$ | 15 | 36 | 32 | 7 | 75B |
| | 30 | 40 | 34 | 8 | 82B |
| | 45 | 43 | 36 | 8 | 87B |
| | 60 | 46 | 37 | 9 | 92B |
| $T_2$ | 15 | 37 | 32 | 7 | 76A |
| | 30 | 41 | 34 | 8 | 83.5A |
| | 45 | 44 | 36 | 8 | 88.5A |
| | 60 | 47 | 37 | 9 | 93.5A |
| $T_3$ | 15 | 34 | 31 | 7 | 73.5C |
| | 30 | 37 | 33 | 8 | 78.5D |
| | 45 | 40 | 35 | 8 | 83.5D |
| | 60 | 43 | 36 | 9 | 88.5D |

*Means of the total acceptability scores with the same letter among treatments for each ripening period are not significantly different ($p \leq 0.01$).

** see Table (2)

## CONCLUSION

From the foregoing results, it could be concluded that using freeze-shocked *Lb. helveticus, Lb. casei* and *Lb. plantarum* as adjunct culture for Edam cheese making has a pronounced effect on both flavour and body & texture of the resultant cheese and accelerate the ripening period to 45 days instead of 60 days for control cheese. Using *Lb. helveticus* as adjunct culture at 1% on cheese curd improved the cheese quality more effectively than other treatment.

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# أثر استخدام بادئات مساعدة معاملة بصدمة التجميد على جودة جبن الايدام خلال التسوية

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أملا فى تحسين تسوية جبن الأيدام باستخدام 1% بادئ مساعد من جنس *Lactobacillus* المعامل بصدمة التجميد (-18 °م/ 96 ساعة) لخثرة جبن منسوبا الى كمية اللبن الداخلة فى صناعه الجبن مع تتبع أثر تلك الأضافة على صفات الجودة. تم صناعه عينة المقارنة باستخدام البادئ التقليدى فقد. بينما باقى معاملات الجبن الثلاث تم استخدام البادئ التقليدى مع 1% من بادئات *Lb. casei & Lb. helveticus & Lb. plantarum* لخثرة الجبن على الترتيب لانتاج المعاملات الثلاث. وتم تتبع التغيرات في التركيب الكيماوي، الصفات الريولوجية، دلائل التسوية المختلفة والتقييم الحسي للمعاملات المختلفة خلال 60 يوما من التسوية. أشارت النتائج الى ان معامله خثرة الجبن بالبادئ المساعد لم يكن له تأثير معنوى على التركيب الكيماوى للجبن سواء من حيث الرطوبة، الدهن فى المادة الجافة، البروتين الكلى، الملح والملح فى الوسط المائى مقارنة بعينه المقارنة. بينما كان لاستخدام تلك السلالات تأثير واضح على زيادة قيم دلائل التسوية معبرا عنها بالنتروجين الذائب منسوبا إلى النتروجين الكلى، النيتروجين الغير بروتيني منسوبا للبروتين الكلى، قيم الأحماض الامينية الحرة الكلية و قيم الأحماض الدهنية الطيارة الكلية مقارنة بعينه المقارنة. وكانت أفضل المعاملات تلك المصنعة باستخدام بادىء *Lb. helveticus* وتلاها تلك المعامله بسلاله *Lb. casei*. أدى استخدام البادئات المساعدة الى خفض قيم الصفات الريولوجية للجبن الناتج خاصة فى نهاية التسوية لقيم اقل من عينة المقارنة. حازت معاملات الجبن المصنعه باستخدام البادئ المساعد على درجات تقييم حسى اعلى من عينه المقارنة وكان القبول الكلي لها افضل وكان لاستخدام تلك السلالات اثر واضح فى اسراع تسوية الجبن ل 45 يوما بدلا من 60 يوما لعينة المقارنة وكان افضل تلك السلالات هى تلك المصنعه باستخدام بادىء *Lb. helveticus* وتلاها تلك المعامله بسلاله *Lb. casei*. وكانت المعاملة الأعلى تأثيرا هى تلك المصنعة باستخدام 1% بادىء *Lb. helveticus* المعامل بصدمة التجميد حيث اعطت جبن أيدام على درجة جودة عالية مع اسراع تسويتها لتكون 45 يوما بدلا من 60 يوما مقارنة بعينة الكنترول.

***الكلمات المفتاحية:*** جبن الأيدام – البادئات المعاملة بصدمة التجميد- الخواص الريولوجية – دلائل التسوية- الخواص الحسية.