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# Microbiological and Chemical Properties of Gravier Cheeses

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**SUMMARY:** In this study, a total of 9 gravier cheese samples in Kars were examined for their chemical and microbiological qualities. Microbiological analysis determined that total mesophilic aerobic bacteria was $2.3x10^7$ cfu/g, lactic bacteria $5.1x10^5$ cfu/g, lactococ bacteria $8.5x10^6$ cfu/g, coliform bacteria $1.0x10^3$ cfu/g, staphylococ-micrococ $4.8x10^4$ cfu/g, sulpite reducing clostridia $4.2x10^2$ cfu/g and yeast and mould $3.9x10^4$. *E.coli, S.aureus* and *C.perfringens* were detected as $<1.0x10^2$ cfu/g . In chemical analysis, amounts of (mean, %) moisture, fat, protein, salt, ash, and acidity were 19.8, 32.4, 31.3, 11.0, 5.4 and 1.54 LA respectively.
The results of the analysis indicated that, 76.6% of gravier cheese samples were found to be incompatible as compared to the Turkish Food Codex's microbiologic criteria. Therefore, gravier cheese sold in the markets in Kars is thought that it may carry a potantial risk factor for public health.
**Key words**: Cheese, Gravier, Microbiological and Chemical quality

**Gravyer Peynirinin Mikrobiyolojik ve Kimyasal Özellikleri**

**ÖZET:** Bu çalışmada, Kars piyasasında satışa sunulan gravyer peynirlerinin mikrobiyolojik ve kimyasal kalite niteliklerinin ortaya konularak yasal mevzuata uygunlukları araştırılmıştır.
Gravyer peynirlerin mikrobiyolojik analizleri sonucunda (ortalama); toplam aerob mezofil bakteri $2.3x10^7$ kob/g, laktik bakteri $5.1x10^5$ kob/g, laktokok bakteri $8.5x10^6$ kob/g, koliform grubu bakteri $1.0x10^3$ kob/g, stafilokok-mikrokok $4.8x10^4$ kob/g, sülfit indirgeyen klostridia $4.2x10^2$, maya ve küf $3.9x10^4$ düzeyinde izole edilmiştir. *E.coli, S.aureus ve C. perfringens* $<1.0x10^2$ kob/g olarak saptandı.. Kimyasal analizler sonucunda ise (ortalama); rutubet miktarı % 19.8, yağ % 32.4, protein % 31.3, tuz % 11.0, kül % 5.4 ve asitlik % 1.54 LA olarak bulunmuştur.
Bu analizler sonucunda, gravyer peynirin %76.6'sının Türk Gıda Kodeksine uygun olmadığı tespit edilmiştir. Bu nedenle, Kars'da marketlerde satılan gravyer peynirin halk sağlığı açısından potansiyel bir risk taşıyabilmektedir.
**Anahtar Kelimeler:** Gravyer, Mikrobiyolojik ve Kimyasal Kalite, Peynir

## INTRODUCTION

Cheese, a milk product presented by several variaties, is made by coagulating, filtering, and processing milk at different manners. Having been known for along time, it is made by different ways in different cultures. It has been estimated that there are 1400 cheese types all over the world (Anon, 2006a). France, Italy, Spain, and Switzerland are among the famous countries regarding the production and variation of the cheese (Anon, 2006b). There are also several kinds in Turkey (Kamber, 2005a). White cheese, Gravier, and tulum cheese compose most of the cheese production in Turkey (Tekinşen, 2000). DIE 2003 has reported that 10 million ton milk is produced yearly in Turkey, 656 thousand ton of which is in Kars (DİE, 2006) where nearly 150 ton gravier cheese is estimated to be produced (Anon, 2006c). It is among the a few European type cheeses produced in Turkey, particularly, Kars and Ardahan in small businesses, other than big

factories. Production is done in small dairies and cheese produced is sent to big cties where it is mostly consumed.

Gravier cheese originated from Switzerland was brought to Kars by Russians in 1905 (Ulutaş et. al, 1993). It is among the hard cheese group, contains very strong arome, requires too much effort for production, and is one of the 18 cheese types which possess very high economic value in the world (Yöney, 1970). It is also in the same group along with Emmental (Switzerland), Gruyere (France), Fontina (Italy), Samso (Demark), and Gouda and Edam (Netherland) (Adam, 1956). Gravier cheese has its own characteristics: hard, porous, and its shape like a mill stone at 20-60 kg in weight and 40-90 cm in diameter. It is delecious, fatly, and its color is changable from ivory color to strong yellow. It is made from cow's milk in the summer months (June-July) (Kamber, 2005a).

Several reports have amply documented micobiological and chemical characteristics of the white cheese, kashar, and tulum cheese, all frequently consumed in Turkey. However, very few reports have revealed features of the gravier cheese (Adam, 1956; Ulutaş et. al. 1993). This study, therefore, intented to document the microbiological and chemical qualities of the Gravier cheese consumed in Turkey, and to indicate whether it carries the requirements of the laws.

## MATERIAL and METHOD

### Material

A number of 9 Gravier cheese samples sold in Kars were used as the material. Such low number of the samples was due to the fact that there are very few company producing this cheese, producing process takes very long time, and it is produced and sold as very large forms.

### Microbiological analysis

Each sample weighing 10 g was homogenized with 90 ml of 0.1 % pepton water in the stomacher (Bagmixer). The samples later were dilutedwith 0.1 % pepton water at 10 fold, and were inoculated to the selective relevant agars by either drop (0.05ml) or spreading (0.1 ml) (APHA, 1992) methods.

Total aerob mesophile bacteria counts: Plate counts agar (OXOID CM325) was used to count the total aerob mesophile bacteria. Drop plaque inoculation performed petries, were incubated at 37°C for 24-48 h, and colonies observed were counted (OXOID, 1990).

Lactic bacteria counts: MRS agar (OXOID CM361) suggested by Deman et al. (1960) was used for the lactic bacteria counts. Double layer inoculated plaques by drop method were incubated 35ºC for 48-72 h, and colonies observed were counted.

*Lactococci* spp. counts: M17 agar (DIFCO 1857-17) with 10% steril lactose was used for *Lactococcus* counts. Plagues inoculated by drop methods were incubated aerobically at 35°C for 48-72 h, and colonies observed were counted (Terzaghi and Sandine, 1975).

Coliform bacteria and *E. coli* counts: Violet Red Bile Agar (OXOID CM107) was used for coliform bacteria counts. Plagues inoculated by spreading method were incubated an anaerobically at 37°C for 24-48 h, and colonies observed as 1-2 mm in diameter, presipiting the bile, displaying red zones on their arounds, and dark red in color, were counted as coliform bacteria colonies. Then, *E. coli* was defined by IMVIC tests (Indol, Methyl red, Voges Proscauer and Citrate) applied to the 5 randomly selected typical colonies (Harrigan and McCance, 1976).

*Staphylococci- Micrococci* spp. and *S. aureus* counts: Baird Parker Agar (OXOID CM275) with Egg Yolk Tellurite Emulsion (OXOID SR54) was for used for *Staphylococci* and *Micrococci* spp. counts.

Spreading plague method performed petries were incubated aerobically at 37°C for 48 h, and colonies observed were counted as *Micrococci-Staphylococci* spp. colonies. For the *S. aureus* identification, coagulase plasma (in tube), and thermonuclease (DNase) tests were performed on the randomly selected 5 typical colonies (blacke, bright, convex, 1-1.5 mm in diameter, displaying narrow and white belts and zone on their arounds). Positive results were counted as *S. aureus* (OXOID, 1990).

Sulphite reducing *Clostridia* spp. and *C. perfringens* Counts: Perfringens Agar (OXOID CM587) supplemented by OXOID SR93 containing canamycine and polymixin was used for sulphite reducing clostridia spp. counts. As a

second layer, same agar was put in the spreading plague method performed petries. Then, the petries were incubated in the jars where anaerobic environment was provided by adding Gas-Pack (OXOID BR 078), at 37°C for 24-48 h. The observed black colonies at 1-2 mm in diameter were counted as sulphite reducing clostridias. Thus, *C. perfringens* was identified by applying biochemical tests (motilite, indol, lactose gelatine, and nitrate) to the randomly selected 5 typical colonies (OXOID, 1990).

Yeast and Moulds Counts: Rose Bengal Agar (OXOID CM549) with chloramphenicol antibiotic supplement (OXOID SR78) was used for yeast and mould counts. Drop plague method performed petries were incubated aerobically at 25°C for 5 days. Typical colonies observed were counted as yeast and moulds (OXOID, 1990).

**Chemical analysis**:

Protein of the Gravier cheese samples was determined by Macro Kjeldahl (IDF, 1964a), moisture by Dry owen method (IDF, 1982b), fat by Gerber method (APHA, 1992), salt by Mohr method (Fox, 1963), ash by owen method (AOAC, 1990), and acidity by standart method suggested in TS 591 (TSE, 1995a). Samples were worked duplicately.

**RESULTS and DISCUSSION**

Micobiological and Chemical analysis of the Gravier cheese sold at retail stores in Kars were displayed in Table 1 and 2, respectively. Microbiological analysis determined that mean aerob mesophyl bacteria level was $2.3x10^7$ cfu/g (Min $2.6x10^6$-Max $8.0x10^7$), lactic bacteria $5.1x10^5$ cfu/g (Min $3.1x10^4$-Max $1.6x10^6$), *Lactococci* spp. $8.5x10^6$ cfu/g (Min $1.1x10^5$-Max.$4.8x10^7$), coliform bacteria $1.0x10^3$ cfu/g (Min<$1.0x10^2$-Max $4.8x10^3$), *Staphylococci-Micrococci* spp. $4.2x10^4$ cfu/g (Min <$2.0x10^2$-Max $2.2x10^5$), sulphite reducing clostridia $4.2x10^2$ cfu/g (Min <$1.0x10^2$-Max $3.8x10^3$), and yeast and moulds $3.9x10^4$ cfu/g (Min $1.3x10^3$-Max $9.0x10^4$). *E.coli, S.aureus* and *C.perfringens* were detected as <$1.0x10^2$ cfu/g in the samples.

Chemical analysis of the Gravier cheese samples documented that mean moisture level was 19.8 % (Min 16.1-Max 24.2), fat 32.4% (Min 27.3-Max 36.1), protein 31.3% (Min 29.0-Max 34.7), salt 11.0 % (Min 5.9-Max 14.6), ash 5.4 % (Min 4.9-Max 5.9), and acidity 1.54 % LA (Min 1.35-Max 1.84).

**TABLE 1.** Microorganism levels of the Gravier cheese samples (cfu/g).

| Sample No | Aerob mesophile bacteria | Lactic bacteria | *Lactococci* spp. | Coliform | *Staphylococci-Micrococci* spp. | Sulphite reducing *Clostridia* spp. | Yeast and Moulds |
|---|---|---|---|---|---|---|---|
| 1 | $8.0x10^7$ | $1.6x10^6$ | $4.8x10^7$ | $2.4x10^3$ | $3.8x10^4$ | <$1.0x10^2$ | $8.2x10^4$ |
| 2 | $3.6x10^7$ | $7.0x10^5$ | $6.8x10^6$ | $3.2x10^2$ | $5.2x10^3$ | <$1.0x10^2$ | $6.0x10^4$ |
| 3 | $9.8x10^6$ | $4.0x10^5$ | $7.0x10^6$ | $2.0x10^2$ | <$2.0x10^2$ | $3.8x10^3$ | $1.3x10^4$ |
| 4 | $5.4x10^7$ | $5.7x10^5$ | $3.6x10^6$ | <$1.0x10^2$ | $6.0x10^3$ | <$1.0x10^2$ | $1.3x10^3$ |
| 5 | $7.6x10^6$ | $1.8x10^5$ | $3.9x10^6$ | $4.8x10^3$ | $2.0x10^2$ | <$1.0x10^2$ | $9.0x10^3$ |
| 6 | $3.5x10^6$ | $1.4x10^5$ | $4.9x10^6$ | <$1.0x10^2$ | $2.2x10^5$ | <$1.0x10^2$ | $1.7x10^4$ |
| 7 | $7.5x10^6$ | $3.2x10^5$ | $1.2x10^5$ | $2.9x10^2$ | $1.3x10^3$ | <$1.0x10^2$ | $4.7x10^4$ |
| 8 | $2.6x10^6$ | $3.1x10^4$ | $1.1x10^5$ | $1.0x10^2$ | $1.8x10^3$ | <$1.0x10^2$ | $3.9x10^4$ |
| 9 | $1.2x10^7$ | $7.0x10^5$ | $2.0x10^6$ | $1.2x10^3$ | $2.0x10^5$ | <$1.0x10^2$ | $9.0x10^4$ |
| Mean | $2.3x10^7$ | $5.1x10^5$ | $8.5x10^6$ | $1.0x10^3$ | $4.8x10^4$ | $4.2x10^2$ | $3.9x10^4$ |
| Min | $2.6x10^6$ | $3.1x10^4$ | $1.1x10^5$ | <$1.0x10^2$ | <$2.0x10^2$ | <$1.0x10^2$ | $1.3x10^3$ |
| Max | $8.0x10^7$ | $1.6x10^6$ | $4.8x10^7$ | $4.8x10^3$ | $2.2x10^5$ | $3.8x10^3$ | $9.0x10^4$ |

**TABLE 2.** Chemical analysıs of the Gravier cheese samples (%)

| Sample No | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | Moisture | Fat | Protein | Salt | Ash | Acidity (LA) |
| 1 | 23.2 | 33.3 | 30.3 | 8.0 | 5.2 | 1.60 |
| 2 | 24.2 | 27.3 | 29.5 | 13.4 | 5.6 | 1.59 |
| 3 | 22.3 | 33.6 | 32.6 | 5.9 | 5.6 | 1.51 |
| 4 | 19.1 | 31.3 | 31.8 | 12.1 | 5.7 | 1.55 |
| 5 | 20.9 | 32.6 | 29.0 | 12.2 | 5.3 | 1.35 |
| 6 | 16.7 | 36.9 | 30.5 | 10.0 | 5.9 | 1.51 |
| 7 | 17.9 | 32.5 | 34.7 | 9.8 | 5.1 | 1.48 |
| 8 | 18.3 | 32.9 | 31.2 | 12.6 | 5.0 | 1.84 |
| 9 | 16.1 | 32.1 | 32.3 | 14.6 | 4.9 | 1.50 |
| Mean | 19.8 | 32.4 | 31.3 | 11.0 | 5.4 | 1.54 |
| Min | 16.1 | 27.3 | 29.0 | 5.9 | 4.9 | 1.35 |
| Max | 24.2 | 36.1 | 34.7 | 14.6 | 5.9 | 1.84 |

Gravier cheese is one of the western originated cheese types produced in Turkey. It is produced mainly in small dairies in Kars, and is sold and consumed in big cities.

Up to the date, no literature report has documented microbiological aspects of the Gravier cheese in Turkey even though those of the kashar cheese which possesses similiar characteritics have been revealed. For this reason, the results of this study were compared to those of the studies done on Kashar. The level of the total aerob mesophyle bacteria in this study ($2.3x10^7$ cfu/g) shows similarities with the findings of Kamber (2005b), Demirci and Draman (1990) ($4.5x10^3$ cfu/g, $3.6x10^3$ cfu/g), and Kıvanç (1989) in kashar. Likewise, the level of the coliform bacteria ($1.0x10^3$ cfu/g) found in this study was similiar to those of the Kamber (2005b), and Demirci and Draman (1990) ($4.5x10^3$cfu/g and $3.6x10^3$ cfu/g, respectively), but higher than that of the Kıvaç (1989) ($4.2x10^2$ cfu/g) all done on kashar. Moreover, the level of the lactic bacteria found in this study was significantly higher than that of the Kıvanç (1989) ($3.7x10^2$ cfu/g). Kamber (2005b) has reported the level of the sulphite reducing anaerobs in kashar to be $2.0x10^2$ cfu/g (3.3%) while this study has found it on the Gravier cheese to be $4.2x10^2$ cfu/g. Presence of these bacteria in all the samples might be due to the fact that clostiridia is among the microphlora of the hard cheese. The lack of the *C. perfringens* in all the samples indicates that those bacteria found are among the natural microphlora of this cheese type. They were not contaminated externally.

Finally, the yeast-mould level ($3.9x10^4$ cfu/g) found in this study was in parallel with those of the Demirci and Draman (1990), and Kıvanç (1989) ($2.8x10^4$ cfu/g and $4.4x10^4$ cfu/g, respectively) while lower than that of the Kamber (2005b) ($1.3x10^6$ cfu/g).

Table 3 displays the legal levels of the microbiological and chemical characteristics. As can be drawn, 66.6% of the samples analyzed did not reach the TS 2471 Gravier criteria (TSE, 1995b) for coliform bacteria, and 100 % of the samples for the yeast and mould. Again, 66.6% of the samples were inadequate for coliform levels in accordance with the Turkish Food Codex-(TFC) 24511 (Anon, 2001d) criteria. The maximum limit for yeast-mould level in cheese is indicated to be $1.0x10^3$ in TFC while the expression ‘‘the yeast-mold counting is not performed in the cheese maturated by yeast and/or mould’’ is present in it. The results hereby have documented that 66.6% of the Gravier cheese analyzed don’t carry the the requirements of the TFC, and 100% of the samples don’t reach the TS Gravier standarts.

In the interpretation of the results of the microbiological analyses, the high levels of the total bacteria and the presence of the coliform bacteria which is a hygiene indicator, in the Gravier samples are thought to be due to inadequate pastorization of the milk instead of the bad hygienic conditions, during the production periods.

**TABLE 3**. Microbiologicall and chemical features of the Gravier cheese samples and legal status

| | Criteria | Standarts TS 2174 | | | TFC 24511 | | |
|---|---|---|---|---|---|---|---|
| | | Levels | n | % | Levels | n | % |
| Microbiologically | Coliform | 100 adet (max) | 6 | 66.6 | 95 (max) | 6 | 66.6 |
| | *E. coli* | 0 | 0 | 0 | 0 | 0 | 0 |
| | *S. aureus* | 0 | 0 | 0 | 100 (max) | 0 | 0 |
| | *C.perfringens* | 0 | 0 | 0 | 100 (max) | 0 | 0 |
| | Yeast & Moulds | 100 adet (max) | 9 | 100 | - | - | - |
| Chemically | Moisture | 38% (max) | 0 | 0 | Not evaluated since no criteria is found for Gravier cheese. | | |
| | Fat | 45% and 30% (min) | 0 - 1 | 11.1 | | | |
| | Salt | 3-10% | 5 | 55.5 | | | |

Absence of the pathogen microrganisms in the samples including *E. coli*, *C perfringens* and *S. aureus*, all being very essential pathogens in food intoxications, supports this idea. Very high level of the yeast and moulds in the samples is also thought specifically to be because of the bad maturation and sale conditions. Nevertheless, the yeast and mold criteria in TS 2174 (100 per g) is very difficult to achieve since mould plays important role in the maturation of the Gravier cheese. TFC indicates, in any case, that the yeast and mould level is not measured in the cheese whose maturation is provided by them. Accordingly, there seems to be a contradiction between the standarts and TFC.

Moisture level was determined to be between 16.1% , and 24.2%, being average of 19.8%. The variation on the moisture is due to the differences in the milk, methods, salt rates, and duration of the maturation. The moisture level in the cheese is parallel with the level of the dry matter. In the other words, moisture increases as dry matter increases or vice versa. The dry matter shows the nourishment value of any given cheese. Accordingly, the Gravier cheese is the highest dry matter containing cheese type. The moisture level of the Gravier cheese in this study was found to be lower than those of the Ulutaş et al. (1993) (31.8%), and Adam (1956) (31.2%). It was also much lower than those of the other cheeses displayed in the Table 4. TS2174 indicates the maximum moisture level in the Gravier cheese to be 38%. In other words, it requires the dry matter to be 62% at least. All the samples analyzed reached these criteria.

**TABLE 4.** Chemical contents of the Gravier and other same types cheese

| Cheese type | Moisture | Fat | Protein | Salt | Ash |
|---|---|---|---|---|---|
| Emmantal (Reinbold, 1973) | 35.5 | 30 5 | 27.5 | 1.2 | 3.5 |
| Gruyere (Kosikowski, 1981) | 34.5 | 30.0 | 30.0 | 1.1 | 4.1 |
| Samsoe (Kosikowski, 1981) | 39.6 | 27.0 | 26.8 | 1.7 | 3.7 |
| Gouda (Kosikowski, 1981) | 41.0 | 28.5 | 26.5 | 2.0 | 3.0 |
| Fontina (Kosikowski, 1981) | 42.8 | 25.5 | 24.2 | 1.1 | 3.2 |
| Edam ((Kosikowski, 1981) | 43.0 | 24.0 | 26.1 | 2.0 | 3.0 |
| Gravyer (Adam, 1956) | 31.2 | 28,6 | 33,2 | 1,7 | 4.1 |
| Gravyer (Ulutaş et al., 1993) | 31.8 | 33.4 | 28.9 | 3,7 | 1.7 |

Fat level was measured to be between 27.3% and 36.1 %, averaging 32.4%. The broad differences in the fat levels are because of the fact that milk is collected from different sources, and is processed by different techniques. The approximate fat level found in the study is higher than those of cheese types in the same category, shown in Table 4. Moreover, it was in parallel with the results of the Ulutaş et al. (1993) (33.4%), and higher than findings of the Adam (1956) (28.6%).

Protein level was betwen 29.0% and 34.7%, averaging 31.3%. It was higher than those of other cheese type displayed in Table 4, and that

of the Ulutaş et al. (1993) while lower than that of the Adam (1956) (28.9%).

Salt is added to cheese to augment duration and to give flavour and texture. Salt levels vary from sample to sample since bittern concentration prepared for cheese differs greatly and cheese blocks absorb different amount of salt when different amounts of cheese are put into the salting pool. The salt level (11%) determined in this study was found to be 2-3 fold higher than those of he Ulutaş et al. (1993) (3.7%) and Adam (1956) (1.7%). Such high level in the salt is also one of the causes of low moisture level observed in this study.

Ash level in the Gravier samples analyzed is between 4.9% and 5.9%, with a mean value of 5.5%, as seen in Table 2. It contains essential minerals which are very important for nourishment. The differences in the ash level among the samples are because of the fact that there is not such a standart for cheese production, that duration of the maturation and salt levels added differ greatly, and that, consequently, dry matter levels display big variations. The ash level found in this study is higher than those of the other cheese types depicted in Table 4 while in parallel with the findings of the Adam (1956) and Kosikowski (1981) (Gruyere, 4.1%).

Mean acidity level of the gravier cheese samples of this study is, in general, lower than those of the studies reported before.

As the chemical analyses are concerned, there is no significant variation among the results of the samples. On the other hand, they differ greatly as compared to those of the same type of cheese world wide, such as dry matter levels. Although the chemical contents are important criteria to determine the quality ofany given cheese, there is no such indication on the Gravier cheese in the TFC. It is explained only in TS 2174 (Table 3). According to the criteria mentioned hereby, 55.5% of the gravier samples did not provide salt criteria. Moreover, 88.9% of the samples were accepted as fatty Gravier, and 11.1% of them were determined to be out of the standarts mentioned.

In summary, 66.6% of the Gravier samples studied in this research do not provide the criteria mentioned in the TFC, in terms of the microbiological and chemical contents. This value is 100% according to the TS 2174 standarts.

The results indicate that Gravier cheese, contributing mainly to the eceonomy of the Kars, is not produced in accordance with suggested criteria. This leads to the gravier cheese production at bad quality. Hence, production, marketing, and sale conditons seem to be inadequate, and need to be improved. To acquire these milk should be pastorized, cheese production techniques should be standardized, hygiene should be of priority during the all procedures, marketing and sale conditions should be improved, and production should be packed in small packages. Small dairies should also be modernized to produce good quality cheese. Finally, local producers need to be informed on the newly legislated legal rules of the cheese production to achieve a standart.

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