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The effect of milk type and heat treatment on physicochemical and microbiological properties of Tulum cheese whey

Die Wirkung von Milchart und Wärmebehandlung auf die physikalisch-chemischen und mikrobiologischen Eigenschaften der Käsemolke von Tulumkäse

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Summary

In this study, Tulum cheese produced by using cow, sheep, goat milk and mixtures of them and physicochemical and microbiological properties of these cheeses whey were analyzed. Two different methods were used in the cheese production. In the first method, traditional production type was used; for this reason, raw milk wasn't heated. In the second method, heat treated milk (60±2 °C 10 min.) was used. Dry matter was determined to be less in whey of cheeses produced from heat treated milk. It was determined that there were increases in fat losses with whey in cheeses that were produced from sheep and goat milk. Lowest protein content in dry matter was determined to be in whey of heat treated cow-sheep milks (50:50). Color values of whey significantly varied according to heat treatments among milk groups. The highest yeast-mold number was determined in whey of cheeses produced from raw sheep's milk. Lactococci count in whey varied between 2.38–6.35 log cfu/g in mesophilic flora, between 4.26–5.70 log cfu/g in termophilic flora. The lowest enterococci count was found in whey of cheeses made of cow milk.

Keywords: Tulum cheese, Whey, Microbiological properties

Zusammenfassung

In dieser Studie sind die Molkeeigenschaften von Türkischer Tulumkäse untersucht worden, die aus Kuhmilch, Schafsmilch, Ziegenmilch und die Vermischung dieser Milcharten hergestellt wurden. Zwei verschiedene Methoden wurden in der Produktion verwendet. Die erste Methode basiert auf traditionelle Herstellungsverfahren. Bei dieser Methode wurde die Rohmilch zu Tulumkäse verarbeitet. Bei der zweiten Methode ist Wärme behandelte (60±2 °C 10 min.) Milch verwendet worden. Trokkenmasse, Rate der Molke von Wärme behandelten Milch hergestellte Tulumkäse, waren niedriger. Bei den Tulumkäsen die aus Schaf-und Ziegenmilch Mischungen hergestellt sind, ist Fettverlusterhöhung mit Molke festgestell worden. Das niedrigste Eiweiß in Trockenmasse ist bei Molke der Wärme behandelten Kuh-Schafmilchmischung (50:50 %) festgestellt. Farbwerte der Molkeproben haben signifikante Unterschiede zwischen den Gruppen, je nach der thermischen Behandlung. Die höchste Anzahl von Hefen und Schimmelpilzen sind, bei Molke proben von Tulumkäse aus roher Schafsmilch hergestellt wurden, identifiziert. Laktokokke Inhalt der Molke proben haben bei Mesophyllen Flora von 2.38 bis 6.35 log (cfu/g) und bei thermophilen Flora von 4.26 bis 5.70 log (cfu/g) geändert. Die niedrigste Enterokokke Inhalt ist bei Molke von Kuhmilch hergestellte Tulumkäse identifiziert.

Schlüsselwörter: Tulumkäse, Molke, mikrobiologische Spezifikationen

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Introduction

It is known that there are over 50 cheese varieties in Turkey (Bostan 1991). Tulum cheese is the most produced cheese after white cheese and Kasar cheese in Turkey and it has a higher economic value when compared to them (Cakmakci et al. 2008).

Tulum cheese is made from raw milk. Although it is a popular cheese variety, unfortunately there is no standard production technique for it. In many regions of Turkey, Tulum cheese is produced with traditional methods and marketed by villagers in local markets. On the other hand, production in very few dairy plant is carried out with modern methods (Aygun et al. 2005, Oksuztepe et al. 2005).

Tulum cheese production in Turkey is carried out in two types that are considerably different from one another in terms of production techniques and appearance, namely dry and brined. The dry Tulum cheese is produced in larger quantities than the brined Tulum cheese (Kılıc et al. 1998).

Tulum cheese can be made from whole, semi skimmed or non-fat sheep's, goat's, cow's or buffalo's milk or a combination of them. Traditionally, Tulum cheese is produced from non-fat milk for using families (after butter production) and sometimes yoghurt can be added to give flavor (Erdogan et al. 2003, Gurses et al. 2003). Tulum cheese is characterized as; white or cream colored, high dry matter, fat and protein ratio, not easily broken, medium-hard, homogenous textured with a characteristic taste and melts in the mouth revealing an easily felt original butter taste (Kurt et al. 1991).

Tulum cheese may show varying physicochemical content. Some researchers (Akyuz 1981, Kurt et al. 1991, Bostan et al. 1992) have recorded that dry matter, fat and lactic acid range from 39.11 to 72.70 %, from 13.30 to 41.0 %, and from 0.612 to 3.100 %, respectively. The volatile flavour compounds in Tulum cheese was studied by Hayaloglu et al. (2007) who identified about 100 volatiles in 90 d-old cheese. The main components were short-chain fatty acids, 2-butanone, diacetyl and primary alcohols, including ethanol.

The microbiological, chemical, and physical characteristics of Tulum cheese depend on the quality of raw milk, the procedures and the conditions of production, experience of the personnel and the storage conditions. As a result, a single standard quality of product cannot be produced (Bostan 1991, Patır et al. 2000). The use of different types of milk (cow's, sheep's or goat's milk) in the manufacture of Tulum cheese was compared by Guven et al. (1995) who reported that the type of milk significantly affected the microbiology of Tulum cheese during 210 d of ripening. In addition to these, Tulum cheese may contain some risks for health (Kurt et al. 1991; Tekinsen and Celik 1979, Akyuz 1981, Kurt et al. 1991). In spite of additional salt, antimicrobial metabolites, low pH and humidity level, some dangerous food pathogens such as *Listeria monocytogenes* and *Salmonella* spp. after ripening period can cause some serious health problems for consumers (Oksuztepe et al. 2005). In order to ensure product safety, some researchers made studies on producing Tulum cheese by using starter culture and pasteurized milk (Arıcı and Simsek 1991, Bostan and Ugur 1992, Sengul 1995).

The name 'tulum' means 'goatskin or sheepskin bag', which is used for packaging and ripening. Although generally cheese is filled into the interior of the tulum. It is reported that especially in the Aegean Region, after the hairs on the outside of goat skin are shaved, turned inside out and used that way (Eralp 1974, Yaygın 1971). During the ripening, the goat skin gives the characteristic taste and sharp aroma to Tulum cheese. Sometimes, the cheese is ripened in hygienic plastic containers used instead of goat skin, because plastic containers are inexpensive, available, and prevent the contamination by some hazardous microorganisms (Erdogan et al. 2003).

In literature, there is no detailed study about physicochemical and microbiological properties of whey of Tulum cheese produced from different types of milk and their mixture depending on heat treatment. In this study, Tulum cheese produced by using cow, sheep, goat milk and mixtures of them and physicochemical and microbiological properties of these cheeses whey were analyzed.

Materials and methods

Milks used in the production of Tulum cheeses

Cow milks (C) used in the research were provided from Akbel Dairy Products Co. (Eregli, Turkey) while sheep (S) and goat milks (G) were provided from small scaled milk plants in the same region. Mixtures of these milks in different ratios (100 % C; 100 % S, 100 % G, 50:50 % C-S, 50:50 % C-G, 50:50 % S-G, 40:40:20 % C-S-G) were used in the production of Tulum cheeses.

Production of Tulum cheeses

Flow diagram of Tulum cheeses production was shown in the Figure 1. Firstly, necessary control and analysis of raw milks chosen for cheese production were made. Next stage, determined milk mixtures for cheese production were prepared. Groups, on which heat treatments were going to be made, were heated for 10 minutes at 60±2 °C in double walled stainless steel cheese vessels. After milks for both cheese groups were cooled to 35±1 °C and liquid rennet (Ecoren 200®, 1:16000, Maysa Food Industry, Istanbul, Turkey) was added. For each milk group, rennet amount was calculated in a way that curd reaches cutting maturity at about 80 minutes; it was 10 fold diluted and was added into the milk at the same temperature. The curd was cut into 2 cm^3 piece and and left to rest (30 min). Bags made of cheese cloth were placed onto the cheese vessel and curd was put into bags. Then the curd was filtered for 30 minutes in the cloth bags; after this step, pressed draining was made for 3 hours under 30 g/cm^2 pressure. Curd that was drained were cut as 5–6 kg weight pieces and waited at room temperature for 7–8 hours. In order to remove dissolved matter in water, fresh cheese that was cut as 1–2 kg weight pieces were kept in cold water for 3–4 hours. This process was repeated for 2–3 times by adding cold water over and over. Fresh cheeses that were removed from water were again filtered for 60 minutes. Cheeses were placed into steel mixer vessel and were crumbled into 4 mm^3 pieces and 2.5 % salt was added and mixed homogenously. Salted cheeses were filled into bags made of goat skin (tulum). 2 mm holes were made on the sides and bottom of tulum. Tulum chesees were left to pre-ripening in a cool and shadow place (18 °C, 1 week). Tulum cheeses were ripened for 1 year at 6±1 °C, 80–85 % relative humidity in the cold room.

Die Inhalte sind urheberrechtlich geschützt. Eine Weitergabe an unberechtigte Dritte ist untersagt.

➡ Raw milk
- ⬇ Control and analysis
- ⬇ Preparation of milk mixtures
- ⬇ Pre-heating (35±1 °C)
- ⬇ *Heating (60±2 °C 10 min.)**
- ⬇ *Cooling (35±1 °C)**
- ⬇ Rennet addition
- ⬇ Coagulation (80 min.)
- ⬇ Curd cutting (2 cm³)

⬅ Whey drainage
- ⬇ Curd holding (30 min.)
- ⬇ Curd draining under without pressure (30 min.)
- ⬇ Curd draining under pressure (30 g/cm², 3 h)

⬅ Whey drainage
- ⬇ Fresh cheese cutting into pieces of 5-6 kg
- ⬇ Fresh cheese holding (room temp, 7-8 h)
- ⬇ Fresh cheese holding in cold water (3-4 h)
- ⬇ Cheese draining under without pressure (60 min.)
- ⬇ Milling of cheese (~4 mm³)
- ⬇ Dry salting (2.5%)
- ⬇ Cheese placement into goatskin (tulum)
- ⬇ Pre-ripening (18 °C, 1 week)
- ⬇ Ripening (6±1 °C, 80-85% relative humidity, 1 year)

FIGURE 1: *Flow diagram of Tulum cheeses production (*for heat-treated milk).*

Physicochemical and microbiological analysis of whey samples

In the analysis, whey samples collected in the process starting from curd cutting until the completion of press were used. Total dry matter (gravimetric method), fat (gerber method), acidity (% lactic acid), pH (sentix 42 electrode, WTW 315i pH meter, Germany) (Anonymous 1981) and total nitrogen (IDF 1993) analysis were made on whey samples. Protein amount was determined by multiplying % nitrogen amount with 6.38 factor. Fat and protein amount in dry matter was determined by calculation. Color analyses were made by using CR400 colorimeter (Minolta, Osaka, Japan) (Pinho et al. 2004). Water activity was determined by water activity measurement device (AquaLab 3 TE, USA) according to AOAC (1995). Media and incubation conditions used in the microbiological analysis of whey samples are shown in the Table 1.

Statistical analysis

Data obtained from physicochemical and microbiological analyses of whey samples were statistically analysed using one-way analysis of variance and the means were separated by Duncan's multiple range test (P<0.01) using Costat software (Costat 1990).

Results and discussion

Physicochemical properties of whey samples

Physicochemical properties of milk types used in cheese production and collected whey samples are presented in the Table 2 and Table 3, respectively. Heat treatment caused significant differences between dry matter values of whey (p<0.01). Highest dry matter ratio (8.46 %) was determined in whey of cheeses made of raw sheep milk. Dry matter was determined to be lower in whey made of heat treated milk.

Fat ratio in whey made of raw sheep, goat and cow milk were determined to be respectively 1.13, 0.89 and 0.82 %. It was determined that there was an increase in fat loss with whey of cheeses made of mixtures including sheep and goat milk. Highest fat ratio in dry matter was determined in S-G group. Lau et al. (1989) reported that fat and protein content maintained higher levels at curd in cheeses produced from pasteurized milk clot. Lau et al. (1990) found that when milk was pasteurised at 63 °C for 30 min, 5 % of the whey protein, which they presumed to be β-lactoglobulin, became associated with the casein protein. Sharma and Dalgleish (1994) showed that during heat treatment whey proteins can be incorporated into the fat globule membrane.

While it was determined that whey of raw sheep milk cheese had the highest protein content, lowest values were determined to be in cheeses made of the mixtures including cow milk. The lowest protein content in dry matter was determined to be in whey of cheeses made of heat treated cow:sheep milk mixtures (50:50). Highest protein loss in dry matter was determined to be in groups including sheep milk. In a study on the relation between bacterial development in milk and protein loss with whey and, it was stated that nitrogen loss with whey in cheeses made of fresh raw milk incur about 25–29 % (McCaskey and Babel 1966). It was pointed out by many researchers that pasteurization increase retention rates in curd due to its denaturation

TABLE 1: *Media and incubation conditions used in microbiological analysis.*

Microorganism	Media		Incubation conditions Temperature (°C)	Incubation conditions Time (day)
Total aerobic mesophilic bacteria*	Plate Count agar (Merck, Germany) 5 % NaCl addition		30	2
Total aerobic psychrophilic bacteria*	Plate Count agar (Merck)		5	7
Yeast-mold*	Potato Dextrose agar (Merck) pH 3.5, acidified with 10 % tartaric acid		25	5
Coliform bacteria*	Fluorocult Violet Red Bile agar (Merck) anaerobic conditions		37	1
Staphylococcus sp.*	Baird Parker agar (Merck) supplemented with 5% egg yolk-tellurite (Merck)		37	2
Lactobacilli sp.[1]*	MRS agar (Merck) pH 5.7, anaerobic conditions	Mesophilic Thermophilic	30 42	2 2
Lactococci sp.[1]*	M17 agar (Merck) pH 7.2, aerobic conditions	Mesophilic Thermophilic	30 42	2 2
Enterococci sp.*	Kanamycin Aesculin Azide agar (Fluka, Germany)		37	2
Lipolytic bacteria[†]	Tributyrin agar (Merck) with glyseroltributyrate		30	5
Proteolytic bacteria[‡]	Skim milk agar (Merck)		21	3

[1]: Cycloheximide (LabM; 100 mg/L) was added to prevent the growth of yeast. *: Sharpe ve Fryer, 1965; Harrigan ve McCance, 1966; Terzaghi ve Sandine, 1975; Gobbetti ve ark., 1999. [†]: Anonymous, 1998. [‡]: Marshall, 1992.

TABLE 2: *Physicochemical properties of milk types used in cheese production.*

Properties	Cow	Sheep	Goat
Total solids (%)	10.53±0.17	16.23±0.45	12.14±0.37
Fat (%)	2.57±0.10	4.54±0.18	3.24±0.06
Fat in TS† (%)	24.40±0.55	27.95±0.37	26.66±0.30
Total Nitrogen (%)	0.45±0.01	0.77±0.04	0.58±0.03
Protein (%)	2.84±0.09	4.88±0.23	3.67±0.18
Protein in TS (%)	26.97±0.37	30.07±0.56	30.22±0.55
Tit. Acid (LA %)‡	0.16±0.02	0.21±0.01	0.14±0.01
pH	6.67±0.03	6.62±0.02	6.73±0.04
Water activity	0.978±0.001	0.976±0.000	0.977±0.002
Color values			
L value	84.74±0.72	84.74±0.72	84.74±0.72
a value	−3.92±0.18	−4.37±0.11	−3.28±0.16
b value	6.01±0.54	7.84±0.88	4.84±0.52

†TS: Total solids, ‡: Titratable acidity (% lactic acid)

effect on curd serum proteins, particularly in moderate to severe heat treatment (63–75 °C/5–30 min.) applied to milk with increased retention rates of proteins in curd, in other words, a decrease in the amount of whey passage (Penev and Prodanski 1962, Kosikowski 1966).

Lactic acid values (%) in whey samples were determined to be higher in samples made of raw milk ($p<0.01$). Samples' lactic acid values were determined to be between 0.10 and 0.13 in samples made of heat treated milks. pH values were determined to be lower in whey of heat treated sheep milk mixtures.

Color values of whey significantly varied according to the heat treatment among milk groups ($p<0.01$). Luminosity values (L) were determined to be between 67.94 and 75.43 in whey of cheeses made of raw milk; between 64.17 and 71.61 in whey of cheeses made of heat treated milks. It was determined that there was a decrease in redness value of cheese whey made of heat treated milks.

Whey of cheeses made of heat treated milk (except whey of C-S-G) were more yellow (b value) intensity. It was found that whey of cheeses made of mixtures including sheep milk had higher b value. Lowest b value (2.72) was determined to be in whey of heat treated cow milk.

Water activity values of wheys were similar ($p>0.01$). Lowest water activity was determined to be 0.981, in whey of cheeses produced from heat treated mixture milk (C-S-G); highest water activity was determined to be 0.986, in whey of cheeses produced from raw cow milk.

TABLE 4: *Microbiological properties of milk types used in cheese production.*

Microorganism	Cow	Sheep	Goat
TAMB†	5.69±0.08	5.74±0.26	5.91±0.20
TAPB	3.34±0.28	4.46±0.23	4.56±0.17
YM	5.37±0.14	6.40±0.19	6.18±0.23
Co	3.67±0.49	4.23±0.06	3.84±0.24
S	3.17±0.22	4.41±0.10	3.71±0.13
MLb	6.15±0.15	6.87±0.54	6.49±0.04
TLb	5.57±0.06	5.72±0.38	5.11±0.12
MLc	6.10±0.06	6.36±0.17	6.30±0.08
TLc	5.80±0.08	6.18±0.06	5.42±0.08
Ec	2.68±0.37	3.88±0.16	2.73±0.08
LB	5.69±0.06	6.35±0.07	5.67±0.16
PB	3.73±0.32	4.38±0.13	3.88±0.16

†TAMB: Total aerobic mesophilic bacteria, TAPB: Total aerobic psychrophilic bacteria, YM: Yeast-mold, Co: Coliform bacteria, S: Staphylococcus sp, MLb: Mesophilic lactobacilli, TLb: Thermophilic lactobacilli, MLc: Mesophilic lactococci, TLc: Thermophilic lactococci, Ec: Enterococci sp, LB: Lipolytic bacteria, PB: Proteolytic bacteria

TABLE 3: *Physicochemical properties of whey samples.*

Properties		Milk types used in cheese production						
		Cow (C)	Sheep (S)	Goat (G)	C-S	C-G	S-G	C-S-G
Total solids (%)	R£	7.62±0.04	8.46±0.08	7.30±0.06	7.76±0.07	7.29±0.01	7.45±0.08	7.31±0.04
	HT	7.25±0.08	8.13±0.06	7.12±0.05	7.34±0.05	6.84±0.11	7.07±0.02	6.95±0.02
Fat (%)	R	0.82±0.03	1.13±0.04	0.89±0.01	0.82±0.01	0.85±0.01	1.07±0.02	0.94±0.03
	HT	0.74±0.02	0.92±0.03	0.78±0.01	0.76±0.01	0.74±0.01	0.86±0.01	0.77±0.01
Fat in TS† (%)	R	10.76±0.31	13.30±0.28	12.13±0.09	10.51±0.09	11.60±0.17	14.30±0.14	12.80±0.33
	HT	10.14±0.18	11.26±0.26	10.89±0.12	10.29±0.12	10.75±0.04	12.10±0.16	11.01±0.17
Total Nitrogen (%)	R	0.18±0.01	0.24±0.02	0.20±0.02	0.20±0.01	0.18±0.01	0.22±0.01	0.20±0.01
	HT	0.16±0.01	0.19±0.01	0.17±0.01	0.15±0.01	0.15±0.01	0.17±0.02	0.16±0.01
Protein (%)	R	1.15±0.09	1.50±0.14	1.24±0.14	1.24±0.09	1.12±0.09	1.37±0.09	1.24±0.09
	HT	1.02±0.08	1.18±0.08	1.05±0.09	0.93±0.08	0.93±0.06	1.05±0.14	0.98±0.08
Protein in TS (%)	R	15.02±1.12	17.73±1.42	17.05±1.71	16.04±1.02	15.32±1.20	18.42±1.02	17.03±1.15
	HT	14.07±1.02	14.52±1.00	14.79±1.17	12.61±1.15	13.53±1.11	14.90±1.87	14.24±1.26
Tit. Acid (LA %)‡	R	0.15±0.01	0.17±0.01	0.13±0.01	0.15±0.01	0.14±0.01	0.15±0.01	0.14±0.01
	HT	0.11±0.01	0.13±0.02	0.10±0.01	0.12±0.01	0.10±0.01	0.11±0.02	0.11±0.01
pH	R	6.24±0.03	6.08±0.03	6.12±0.00	6.10±0.01	6.15±0.02	6.12±0.02	6.10±0.01
	HT	6.32±0.02	6.18±0.03	6.26±0.01	6.24±0.01	6.28±0.02	6.20±0.02	6.23±0.01
Water activity	R	0.985±0.001	0.982±0.002	0.984±0.000	0.984±0.001	0.983±0.001	0.983±0.001	0.981±0.003
	HT	0.986±0.001	0.982±0.001	0.985±0.001	0.984±0.001	0.985±0.001	0.984±0.001	0.983±0.002
Color values								
L value	R	71.68±0.80	73.23±0.45	67.94±0.85	68.16±1.83	73.82±0.16	75.43±1.31	73.57±0.69
	HT	71.61±0.12	67.53±0.88	64.25±0.35	64.17±0.32	70.10±0.33	67.78±1.60	69.04±1.03
a value	R	−1.57±0.08	−3.40±0.13	−3.80±0.06	−2.70±0.20	−2.96±0.06	−2.56±0.20	−3.43±0.20
	HT	−3.76±0.04	−4.07±0.16	−4.00±0.12	−4.28±0.09	−3.21±0.08	−2.36±0.18	−3.17±0.05
b value	R	2.72±0.10	5.38±0.20	6.59±0.17	4.78±0.34	4.45±0.06	3.89±0.23	5.80±0.35
	HT	5.81±0.03	7.70±0.14	6.84±0.06	7.15±0.26	4.65±0.10	4.11±0.32	5.32±0.04

†TS: Total solids, ‡: Titratable acidity (% lactic acid), £ R: Raw, HT: Heat treatment

TABLE 5: *Microbiological properties of whey samples (log cfu/g).*

Microorganism		Milk types used in cheese production Cow (C)	Sheep (S)	Goat (G)	C-S	C-G	S-G	C-S-G
TAMB†	R[£]	5.24±0.32	5.68±0.30	6.05±0.17	5.34±0.12	5.41±0.06	5.76±0.20	5.53±0.25
	HT	2.39±0.25	3.14±0.07	3.86±0.39	2.75±0.26	2.90±0.18	3.52±0.20	2.86±0.25
TAPB	R	4.32±0.04	4.85±0.16	4.92±0.30	4.35±0.15	4.46±0.09	4.59±0.12	4.42±0.13
	HT	2.38±0.54	3.08±0.22	3.88±0.05	2.89±0.32	3.24±0.12	3.37±0.25	2.70±0.25
YM	R	6.16±0.24	6.50±0.04	6.40±0.18	6.30±0.12	6.27±0.18	6.36±0.28	5.98±0.32
	HT	2.35±0.20	2.98±0.12	2.58±0.18	2.60±0.05	2.54±0.16	2.78±0.19	2.65±0.16
Co	R	3.84±0.12	4.68±0.40	4.37±0.16	3.90±0.06	4.23±0.11	4.45±0.19	3.96±0.12
	HT	2.29±0.10	2.96±0.06	2.44±0.12	2.45±0.14	2.27±0.26	2.61±0.10	2.56±0.17
S	R	2.98±0.12	3.60±0.25	3.14±0.17	3.36±0.25	3.12±0.07	3.47±0.09	3.22±0.25
	HT	2.05±0.18	2.56±0.16	2.26±0.28	2.28±0.24	2.24±0.18	2.45±0.03	2.08±0.20
MLb	R	6.26±0.18	6.58±0.27	6.35±0.30	6.32±0.15	6.30±0.10	6.43±0.40	6.29±0.47
	HT	2.84±0.20	3.34±0.26	3.16±0.12	3.14±0.47	2.86±0.18	3.25±0.30	3.26±0.33
TLb	R	5.10±0.12	5.47±0.18	4.82±0.15	5.16±0.12	4.99±0.26	5.33±0.32	5.13±0.12
	HT	4.75±0.54	4.82±0.14	4.30±0.20	4.80±0.12	4.53±0.48	4.69±0.16	4.63±0.20
MLc	R	6.15±0.52	6.35±0.04	6.28±0.04	6.21±0.05	6.23±0.18	6.27±0.12	6.20±0.12
	HT	2.38±0.28	3.37±0.18	3.01±0.26	2.78±0.04	2.56±0.29	3.10±0.10	2.50±0.20
TLc	R	5.30±0.14	5.70±0.13	5.48±0.12	5.52±0.35	5.32±0.40	5.65±0.04	5.52±0.09
	HT	4.26±0.69	4.77±0.25	4.49±0.25	4.56±0.45	4.36±0.18	4.67±0.07	4.30±0.19
Ec	R	3.59±0.35	4.37±0.30	4.24±0.42	4.02±0.30	4.10±0.12	4.27±0.04	4.16±0.12
	HT	2.53±0.25	4.14±0.18	3.44±0.02	3.73±0.20	3.29±0.15	3.97±0.18	3.59±0.17
LB	R	5.97±0.20	6.68±0.05	6.54±0.30	6.46±0.12	6.34±0.20	6.58±0.12	6.25±0.32
	HT	5.18±0.04	5.73±0.12	5.38±0.26	5.35±0.24	5.34±0.14	5.56±0.25	5.24±0.28
PB	R	3.64±0.06	4.61±0.18	3.96±0.19	4.11±0.64	3.68±0.15	4.24±0.35	3.76±0.18
	HT	2.98±0.35	3.65±0.06	3.29±0.13	3.28±0.18	3.16±0.20	3.44±0.32	3.15±0.12

[†]TAMB: Total aerobic mesophilic bacteria, TAPB: Total aerobic psychrophilic bacteria, YM: Yeast-mold, Co: Coliform bacteria, S: Staphylococcus sp, MLb: Mesophilic lactobacilli, TLb: Thermophilic lactobacilli, MLc: Mesophilic lactococci, TLc: Thermophilic lactococci, Ec: Enterococci sp, LB: Lipolytic bacteria, PB: Proteolytic bacteria, [£] R: Raw, HT: Heat treatment

Microbiological properties of whey samples

Microbiological properties of milk types used in cheese production and collected whey samples are presented in the Table 4 and Table 5, respectively. There were determined to be significant differences in the whey of cheeses made of different milks in terms of microbiological properties ($p<0.01$).

Total mesophilic bacteria count of samples was determined to be between 2.39 and 6.05 log cfu/g. Aerobic mesophilic flora was decreased in whey of cheeses made of heat treated milk. Total bacteria count of the whey of cheeses made of goat milk was determined to be higher.

In whey of cheeses made of cow, sheep and goat milks, psychrophilic microorganism count are; in cheeses made of raw milk 4.32–4.92 log cfu/g, in cheeses made of heat treated milk 2.38–3.88 log cfu/g. It was determined that heat treatment caused 21–45 % psychrophilic microorganism reduction. Pasteurisation of raw milk is effective in eliminating of microorganisms excluding the thermoduric microorganisms of the genera *Micrococcus, Microbacterium, Streptococcus, Lactobacillus, Bacillus, Clostridium,* the coryneforms, and occasionally some Gram-negative rods (Jay 1996). Therefore, microorganisms present in pasteurised milk are from two major sources, thermodurics and/or postpasteurisation contaminants (Schröder 1984). Thermoduric psychrotrophs can grow at refrigeration temperatures below 7 °C, produce enzymes, toxins and other metabolites (Jay 1996) and contribute to high standard plate counts in both raw and pasteurised milk.

Highest yeast-mold count was determined to be in whey of cheeses made of raw sheep milk. There was determined to be 0.52 log unit difference in whey of cheeses made of raw milk.

Coliform group microorganism count was found to be approximately 4 log unit level in whey of cheeses made of raw milk. Heat treatment significantly reduced coliform group microorganisms and decreased content to 2.5 log unit level. The high level of coliform found in milk used for Tulum cheese production reflects the hygienic condition of milk commonly used for production of Tulum cheese. However, it has been reported that the level of coliform bacteria gradually decreases during ripening of traditional Tulum cheese (Bostan 1991, Ates and Patır 2001).

Highest *Staphylococcus* count (3.60 log cfu/g) was determined in whey of cheeses made of raw sheep milk, lowest count (2.05 log cfu/g) was determined in whey of cheeses made of heat treated cow milk. Bostan (1991) found *Staphylococcus* (coagulase +) number as average 8.4x103 cfu/g in Tulum cheese samples. Digrak et al. (1994) found that 8 out of 17 Tulum cheese samples contained the number of S. aureus below detectable level, while other samples contained $4.8x10^2$–$1.9x10^5$ cfu/g, with an average of $3.5x10^4$ cfu/g.

There was determined to be significant differences in lactic acid bacteria content of whey according to the milk type used production ($p<0.01$). Bostan et al. (1992) experimentally produced Tulum cheese from raw cow milk and isolated a total of 684 strains of lactic acid bacteria at various stages of ripening. Their results showed that *Streptococcus lactis* and *Streptococcus faecalis* were the predominant flora during the initial phase whereas *Streptococcus faecium, Streptococcus lactis, Lactobacillus casei* and *Lactobacillus plantarum* predominated in later stages of the ripening period.

There were determined to be 6.29–6.58 log cfu/g mesophilic lactobacilli in whey of cheeses made of raw milk,

Journal of Food Safety and Food Quality 64, Heft 6 (2013), Seiten 157–188

2.84–3.34 log cfu/g in whey of cheeses made of heat treated milk. Highest reduction of mesophilic lactobacilli was determined in whey of cheeses made of cow milk (54.6 %), while lowest reduction was determined in whey of cheeses made of cow:sheep:goat milk mixtures (48.2 %). Almost 90 % of termophilic lactobacilli survived at the end of heat treatment. It was found that whey of cheeses including sheep milk had the highest thermophilic lactobacilli count. Mesophilic lactobacilli are usually present because of post-pasteurization contamination, but may also constitute a part of the raw milk microflora and may survive pasteurization (De Angelis et al. 2004). Some researchers (Puchades et al. 1989, Lee et al. 1990, Broome et al. 1990, Trepanier et al. 1991) have used mesophilic lactobacilli as adjuncts to the starter with more or less satisfactory results; increased levels of proteolysis (particularly free amino acids) and improved flavour characteristics of the adjunct-containing cheeses were generally observed.

Lactococci count in whey at mesophilic flora was 2.38–6.35 log cfu/g, and at termophilic flora it was 4.26–5.70 log cfu/g. Highest heat treatment reduction in lactococci at mesophilic flora was determined to be in whey of cheeses made of cow milk (61.3 %); at termophilic flora was determined to be in whey of cheeses made of cow:sheep:goat milk mixtures (22.1 %). The predominance of lactococci, which are present in starter culture combination in many types of cheese, is observed in a number of cheese varieties and then the other genera such as enterococci or lactobacilli become dominant in the late ripening period (Lopez-Diaz et al. 2000, Bulut et al. 2005). The species lactococci were at levels of 15.8 % in survey samples of matured Tulum cheese (Oner et al. 2004).

Enterococci count in whey was between 2.53–4.37 log cfu/g. Lowest enterococci number was found in the whey of cheeses made of cow milk. Bostan et al. (1992) that 22 to 33% of isolates were identified as enterococci during the ripening of Tulum cheese produced from raw milk. Relatively high-level presence of enterococci might be due to acid-tolerant and halophilic members of enterococci such as *Streptococcus faecium, Streptococcus faecalis* and *Streptococcus thermodurans.* The role of enterococci in the ripening of cheese, particularly in aroma and flavor development, has not been clearly established yet (Lopez-Diaz et al. 2000).

Lipolytic microorganism count in whey of cheeses made of raw cow, sheep and goat milk was found to be respectively 5.97, 6.68 and 6.54 log cfu/g. Proteolytic microorganism count in whey of cheeses made of raw milk was at 4 log unit level. Highest proteolytic microorganism count was determined in the wheys of cheeses made of sheep milk, lowest proteolytic microorganism count was determined in the samples made of cow milk. Heat treatment caused 14.13 % decrease in the number of proteolytic microorganism in whey of cheeses made of cow:sheep:goat milk mixtures; 20.19 % decrease in whey of cheeses made of cow:sheep milk mixtures.

Conclusion

Heat treatment caused significant differences between dry matter values of whey. It was determined that there was an increase in fat loss with whey of cheeses made of mixtures including sheep and goat milk. Total bacteria count of the whey of cheeses made of goat milk was determined to be higher. Coliform group microorganism count was found to be approximately 4 log unit level in whey of cheeses made of raw milk. The results of this work showed that hygienic quality and heat treatment in milks that are used as raw material in the production of Tulum cheese, provided significant benefits in terms of microbial properties.

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