

## Hygienic Indicators and Chemical Composition of Prgica Cheese Produced from Raw and Pasteurised Milks

KRISTIJAN VALKAJ<sup>1</sup>, SAMIR KALIT<sup>2</sup>, MILNA TUDOR KALIT<sup>2</sup> and WILLIAM L. WENDORFF<sup>3</sup>

<sup>1</sup>Vocational School, Čakovec, Croatia; <sup>2</sup>Department of Dairy Science, Faculty of Agriculture, University of Zagreb, Croatia; <sup>3</sup>Department of Food Science, University of Wisconsin, Madison, USA

### Abstract

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The hygienic indicators and chemical composition of Prgica cheese produced from raw and pasteurised milks as well as the microbiological quality of dry red pepper used in the cheese production were investigated. Prgica cheeses and originally packed dry red peppers were collected from five family farms and five vacuum-packed Prgica cheese samples were purchased from the supermarkets. Significantly lower fat ( $P < 0.01$ ), protein, and total solids contents ( $P < 0.0001$ ) of artisanal Prgica cheese in comparison to Prgica cheese purchased from the supermarkets were detected. Two samples of cheese produced on family farms and three samples purchased from the supermarkets had high numbers of yeasts and moulds. The yeasts *Mucor* sp. and *Candida famata*, and moulds *Aspergillus niger* and *Aspergillus ochraceus*, were detected in dry red pepper. The results showed that there was potential yeast and mould contamination among the Prgica cheeses produced from raw milk, as well as Prgica cheeses purchased from the supermarkets, produced from pasteurised milk under controlled conditions, also contained high numbers of yeasts and moulds probably due to contamination by dry red pepper used in their production.

**Keywords:** yeasts; moulds hygienic quality; dry red pepper

Prgica cheese belongs to the type of sour, dried, cone shaped cheese with salt and dry red pepper added in the cheese body which is produced in the north-western part of Croatia. The name, chemical composition, and size of this cheese type depend on the region where it is produced (LUKAČ-HAVRANEK 1995). It is traditionally made on family farms from fresh sour cheese coagulated by lactic acid fermentation of raw milk with no starter addition. The mixture of sour, fresh cheese and ingredients is formed with hands into small cones (approximately 100–120 g/cone), which are air-dried for two days (LUKAČ-HAVRANEK 1995; VITEZ & MURAJ 2001).

The aim of this study was to investigate the hygienic indicators and chemical composition of artisanal Prgica cheese produced from raw milk

versus Prgica cheese purchased from the supermarkets that is produced from pasteurised milk. The microbiological quality of dry red pepper used in the cheese production was also investigated.

### MATERIAL AND METHODS

**Cheese and dry red pepper samples.** Prgica cheeses and originally packed dry red peppers used for Prgica cheese production were collected from five family farms that sell their cheeses at the local green market. Moreover, five samples of originally vacuum-packed Prgica cheese produced from pasteurised milk were purchased from the supermarkets. The purchased cheeses had different production dates (different batches).

**Cheesemaking of artisanal Prgica cheese.** The artisanal Prgica cheese was produced from sour, fresh cow's cheese, salt, dry red pepper, and garlic. The sour coagulum for draining was obtained by lactic acid fermentation of raw cow's milk with no starter addition. The cheese mixed with spices was used for forming, with hands small cone shapes which were then air-dried. During the summer season, the cheeses were dried outdoors while during the wintertime, they were dried near a wood fuel oven.

Sour, fresh cow's cheese was produced from raw milk soon after milking and filtration on the farm. Milk was put into glass vats (2.5 or 5 l volume) at room temperature (about 22°C). No starter was used. After acid coagulation (after 2–3 days), the separated cream was removed from the surface of the glass vat. Skimmed fermented milk was put into a vat for heating near the oven for 2–3 hours. The temperature of the sour coagulum must be lower than 40°C. The curd was then put into cheesecloth or a cheese bag to drain. After 12 h draining period, the curd in cheesecloth was pressed for 12 hours. During pressing, the moisture content decreased and tall pointed cones were easily formed by hands.

**Chemical and microbiological analyses.** Chemical analyses of cheese were performed at the Reference Laboratory of Dairy Science Department at the Faculty of Agriculture, University of Zagreb. Microbiological analyses of cheese were carried out at the Department of Hygiene and Technology of Animal Products Laboratory at the Veterinary Faculty, University of Zagreb. The analyses of Prgica cheese included the determination of fat content according to the Van Gulik method (HRN EN ISO 3433), protein content according to Kjeldahl method (HRN EN ISO 8968-5), total solids (HRN EN ISO 5534), pH value (Mettler Toledo Seven Multi, according to manufacturer's instructions; ), and salt content according to Volhard (AOAC 935.43). The cheese weight was determined in the family farms using a digital scale FA-6406 (with the accuracy of 1 g). The presence of *Salmonella* spp. (HRN EN ISO 6785) and *Listeria monocytogenes* (HRN EN ISO 11290-1) was considered as food safety criteria, whereas *E. coli* (HRN ISO 11866-1), *Staphylococcus aureus* (HRN EN ISO 6888-1), sulphur-reducing *Clostridium* (HRN ISO 15213), yeasts, and moulds (HRN ISO 6611) were considered as hygienic indicators of the cheese.

The yeasts and moulds in the original packages of dry red peppers used for artisanal Prgica cheese

production were analysed at the Center for Poultry Farming at the Croatian Veterinary Institute in Zagreb according to the method HRN EN ISO 7954. Sabouraud Dextrose Agar (Biolife 4020052) was used for yeasts and moulds counting. The growth of yeasts and moulds took place at 25°C over 5 days. The conformation test was done with api Candida (bioMerieux 10 500; bioMerieux Inc., Durham, USA).

**Statistical analysis.** The statistical analysis was performed using the statistical software SAS Version 9.2 (Institute Inc., Cary, USA) and the GLM procedure. The data are shown as mean  $\pm$  standard deviation. Significant differences between the measurements are reported at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### The composition of cheese

During 2–3 days fermentation period of raw milk for artisanal Prgica cheese, part of milk fat was removed as sour cream due to the cream separation. This resulted in a significantly lower fat content of artisanal Prgica cheese in comparison to Prgica cheese purchased from the supermarket and produced from pasteurised milk (Table 1). Moreover, the contents of proteins and total solids in Prgica cheese produced from pasteurised milk were significantly ( $P < 0.0001$ ) higher than in those produced on family farms. This could be due to the difference between the intensities of the drying procedure applied during the two different manufacturing protocols. Prgica cheese is considered a traditional product, mainly produced on family farms for local green market; however, Prgica cheese purchased from the supermarket was more intensively dried for extending the shelf life and improving the quality. The differences between the salt contents ( $P = 0.113$ ), weights ( $P = 0.101$ ), and dimensions ( $P = 0.074$ ) were not significant (Table 1).

### Microbiological analyses

*Salmonella* spp. and *Listeria monocytogenes* were not detected in 25 g of the cheese. *Escherichia coli*, *Staphylococcus aureus*, and sulphur-reducing *Clostridium* were detected in numbers  $< 10$  CFU/g in all cheeses. Therefore, the results of the micro-

Table 1. Chemical composition and characteristics of artisanal produced Prgica cheese from raw milk versus Prgica cheese produced from pasteurised milk

Parameter	Family farm (mean value $\pm$ SD)	Supermarket (mean value $\pm$ SD)	Significance
Total solids (g/100 g)	39.34 $\pm$ 2.45	56.59 $\pm$ 1.62	$P < 0.0001$
Proteins (g/100 g)	22.09 $\pm$ 2.47	31.43 $\pm$ 1.06	$P < 0.0001$
Fat (g/100 g)	8.80 $\pm$ 4.72	16.0 $\pm$ 1.58	$P < 0.01$
Salt (g/100 g)	2.35 $\pm$ 0.68	1.81 $\pm$ 0.07	NS ( $P = 0.113$ )
pH	4.40 $\pm$ 0.03	4.50 $\pm$ 0.25	NS ( $P = 0.414$ )
Weight (g)	120.40 $\pm$ 24.65	100.00 $\pm$ 0	NS ( $P = 0.101$ )
Height (cm)	9.40 $\pm$ 0.65	10.00 $\pm$ 0	NS ( $P = 0.074$ )
Width (cm)	5.60 $\pm$ 0.55	6.00 $\pm$ 0	NS ( $P = 0.141$ )

NS = non significant

biological analyses of the cheese samples of both groups were in accord with the microbiological criteria applicable to foodstuffs as appeared in the Guide for the Microbiological Criteria for Food (2009). Similar results were obtained by VALNEGRI *et al.* (2011) for artisanal mountain Fatuli cheese. However, two samples of cheese produced on family farms (A and B) and three samples purchased from supermarkets (F, G, J) contained high numbers of yeasts and moulds (more than  $10^3$  CFU/g; Table 2) according to the Guide for the Microbiological Criteria for Food (2009). With the exception of the cheese ripened in controlled conditions with surface moulds and smear, the contamination of cheese with moulds and yeasts is not desirable and is considered to be a fault for all other cheese types (ADDIS *et al.* 2001). Lactic acid bacteria are a normal natural milk microflora and by their metabolical activity, they affect the total quality of sour, fresh cheese. LAB metabolism

results in an acidic environment, in which most of the saprophytic and pathogenic microorganisms cannot survive (ZDOLEC *et al.* 2007). Moreover, fermented milk of a low pH (4.2–4.6) is not the optimal medium for the growth of most spoilage bacteria (ROBINSON *et al.* 2002, 2006), however, it can favour the growth of moulds and yeasts, which can negatively affect the appearance and flavour of cheese (ROBINSON *et al.* 2002).

DOBRANIĆ (2006) determined lower numbers of *E. coli* in 12 days old sour, fresh cheese compared to the sour, fresh cheese just a day after manufacturing. On the other hand, in cheese of the same storage period, higher numbers of yeasts and moulds were isolated. Excessive numbers of yeasts and moulds (more than  $10^3$  CFU/g) were found in the traditional Croatian fresh cheese investigated earlier (Table 3) (LUKAČ & SAMARŽIJA 1990; KOZAČINSKI *et al.* 2003; KIRIN 2009; MARKOV *et al.* 2009).

Our investigation confirmed previous findings (Table 2). Similarly, 85.71% samples of same type kvargl cheese contained excessive numbers of yeasts and molds (KIRIN 2004).

Prgica cheese purchased from the supermarket had been made from fresh cheese manufactured from pasteurised milk. Before fermentation, the milk is pasteurised at 74° C for 40 s, which results in the destruction of all harmful and pathogenic microorganisms (KIRIN 1980). The process of milk pasteurisation significantly reduces the number of microorganisms in fresh cheese, mainly fungi (DOBRANIĆ 2006). This ensures that the pasteurised milk is not a source of yeasts and moulds. Since Prgica cheese purchased from the supermarket was manufactured in strictly controlled conditions, the cause of its contamination should be

Table 2. Mycological quality of Prgica cheese

Microorganisms	Yeasts and moulds (CFU/g)
Farm A	$2.8 \times 10^4$
Farm B	$2.1 \times 10^3$
Farm C	$5 \times 10^2$
Farm D	$6 \times 10^2$
Farm E	$9 \times 10^2$
Supermarket F	$3 \times 10^4$
Supermarket G	$2.5 \times 10^4$
Supermarket H	$5 \times 10^2$
Supermarket I	$2 \times 10^2$
Supermarket J	$7.6 \times 10^3$

Table 3. Hygienic quality of traditional Croatian fresh cheese regarding yields and moulds

Citation	<i>n</i>	(%)*
MARKOV <i>et al.</i> (2009)	60	65.00
KIRIN (2004)	14	100.00
KOZAČINSKI <i>et al.</i> (2003)	127	26.77
LUKAČ & SAMARŽIJA (1990)	13	76.92

\*percentage of samples with regard to high yeasts and moulds count (more than  $10^3$  CFU/g)

in the production practice, i.e. the addition of dry red pepper and drying. The source of contamination with yeasts and moulds could be the contaminating additives (ROBINSON *et al.* 2002, 2006). According to VRAČAR *et al.* (2007), dry red pepper is a spice very susceptible to contamination by microorganisms. Therefore, the estimation of yeasts and moulds counts in dry red pepper used in the experimental production of Prigica cheese at family farms was carried out. The results showed the presence of yeasts and moulds (Table 4). The following microorganisms were found in the samples: the yeast *Mucor* sp. and moulds *Aspergillus niger* and *Aspergillus ochraceus* in the first and second samples, and the yeasts *Candida famata* in the third, fourth and fifth samples (Table 4).

KARAN *et al.* (2005), VRAČAR *et al.* (2007), ŠARIĆ and ŠKRINJAR (2008) detected the presence of *Aspergillus* sp. and *Mucor* sp. in samples of dry red pepper. One of the main sources of yeasts and moulds contamination of Prigica cheese could be dry red pepper. To reduce the initial number of fungi in dry red pepper, the method of short sterilisation has been recommended by VRAČAR *et al.* (2007), reducing the total number of yeasts and moulds by a factor of 160. However, the heat treatment of dry red pepper can cause the loss of the intense red colour. To reduce the negative effect of the heat treatment on the physical properties and chemical composition of dry red pepper and

to obtain a good microbial elimination, the gamma rays radiation (10 kGy) has been recommended by RICO *et al.* (2010).

## CONCLUSION

Chemical composition of Prigica cheese purchased from the supermarkets differed from the Prigica cheese produced in family farms, which clearly showed the differences in the manufacturing procedures. High numbers of yeasts and moulds in Prigica cheese (both in family farm produced and purchased from the supermarkets) were observed. Possible reasons for the cheese contamination during manufacturing may be the addition of dry red pepper containing high numbers of yeasts and moulds.

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Table 4. Mycological analyses of dry red pepper

Sample	Yeasts and moulds (CFU/g)
1	$4.55 \times 10^3$
2	$3.00 \times 10^3$
3	$1 \times 10^4$
4	$3.5 \times 10^3$
5	$2 \times 10^2$

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*Corresponding author:*

Prof. Dr.Sc. SAMIR KALIT, University of Zagreb, Faculty of Agriculture, Department of Dairy Science, Svetošimunska 25, 10000 Zagreb, Croatia; E-mail: skalit@agr.hr

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