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Manufacturing process and physicochemical analysis of Kariki: a traditional cheese from the Island of Tinos, Greece

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Abstract

The present work aimed to identify, for the first time, the traditional manufacturing process and the physicochemical properties of the Kariki cheese originated from the Island of Tinos, Greece. Various physicochemical parameters (moisture, protein, fat and NaCl content, pH, acidity, fatty acid profile, and pigments) were determined on cheese samples (matured for three months in a dried calabash). The samples were obtained from the only dairy company in Tinos Island that produces this type of cheese using traditional methods. The results showed that Kariki is a yellow cheese, hard on the outside but soft on the inside, with low moisture and acidity content, and high proportion of fat comparable to similar types of cheese products. These characteristics are probably derived from the maturation process in the calabash. Kariki also showed much higher content in saturated fatty acids and lower content in monounsaturated and polyunsaturated fatty acids compared to other cheeses with similar maturing time.

Introduction

Milk is the first food of newborn mammals. It is a complete food for humans as it consists of proteins, fats, water, vitamins, minerals, sugars, and salt (O' Mahony and Fox, 2013). Although milk is an integral part of the diet, it has a serious disadvantage; it is perishable. For this reason, humans have invented various methods such as pasteurisation, evaporation, fermentation, and turned milk into various end-products such as butter, yogurt, and cheese in order to increase the shelf life, nutritional values, taste, and digestibility of these products (Roberts *et al.,* 2005).

The preparation of cheese is one of the most classical examples of food preservation. Cheese is a generic name for a group of milk-based fermented products which are produced in a wide range of flavours and forms around the world. Although the primary role of cheese production is to maintain milk components for longer period, cheese has been developed into a product of high gastronomy, and characterised for its high nutritional value. The conversion of milk into cheese helps in the conservation of the most important components of milk (fats and proteins). Cheese production is based on two preservation principles: (i) the fermentation with lactic acid which comes from the inoculation of bacterial colonies or the naturally occurring microflora, which lower the pH, and (ii) the reduction of water activity and the addition of NaCl. The combination of the above contributes to the stability of cheese for a long period of time. Cheese production essentially involves the gelatinisation of casein by means of equimolar (acid) or enzymatic (hard) coagulation. Some cheese types are produced with a combination of heating and acidification, and rarely by thermal evaporation as described by Kindstedt (2012).

Nowadays, around 500 different cheese varieties are produced around the world, giving 10^7 tons of cheese per year. These numbers are rising at a rate of ~4% per year. According to the Eurostat Statistics (2019), in 2016, 656,494 tons of milk originating from cows, and 1,253,659 tons from other animals were produced. Of all the milk-based products, the quantity of cheese was 144,470 tons. According to the data of Hellenic Statistical Authority (2019), in 2016, the southern Aegean region produced 877 tons of soft cheese, and 2,544 tons of hard cheese. From those, Tinos Island produced five tons of soft cheese, and 50 tons of hard cheese.

Kariki cheese, or trachilas (wild, as previously called), or Tinian blue cheese, has been produced since 1880s in Tinos Island, and especially, in the villages of Myrsini, Steni, and Falatados situated on the mountains of Tsiknias. Its production was not widespread to the whole island, but limited only to the villages mentioned above, as the only ways to spread products at that time was through marriages (Kariki

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cheese was offered to guests among other foods after the wedding ceremony) and street vendors. The appearance of the cheese did not encourage its dissemination in this way; so, its use was restricted to the houses where it was produced for household consumption. Therefore, this cheese has remained relatively unknown until today. Kariki cheese could have accidentally originated from another cheese called "petroma". The latter was transported and kept (in order to be protected from flies and insect parasites) in the flask for a long period with the flask ending up as a milking pot. Today, the only known producer of Kariki cheese is a small and traditional family-owned cheese-making factory headed by Mrs. Aggela Rouggeri (Steni Village, Tinos Island, Greece).

Depending on the degree of maturation, Kariki may either resemble Roquefort or Stilton cheese. Its unique feature is that its maturation process takes place in an empty gourd (calabash), a type of pumpkin, for a time period that varies between 40 days and seven months (The House, 2019). Calabash or *Lagenaria siceraria* (*Lagenaria vulgaris* Ser.) or Kariki (as it was initially known in Tinos Island, and whence the name of cheese came from) is one of the oldest cultivated plants, and has been used by humans as a functional or decorative object all over the world (Milind and Kaur, 2011). In order to eat Kariki cheese, one must first remove it from the calabash. It is a yellow cheese, hard on the outside and soft in the inside, and when cut, it crumbles like Parmigiano cheese. It is salty and spicy.

The aim of the present work was to present, for the first time, the traditional manufacturing process of Kariki cheese and describe its physicochemical properties.

Materials and methods

Chemicals

Sodium hydroxide and hydrochloric acid were purchased from Panreac (Polígono Pla de la Bruguera, E-08211, Barcelona, Spain). Nitric acid, *n*-hexane, and sodium chloride were purchased from Merck (Frankfurter Str. 250, 64293, Darmstadt, Germany). Fatty acid methyl esters (FAMEs) mix standard was purchased from Supelco (595 N Harrison Rd, PA16823, Bellefonte, USA). All other reagents (analytical and HPLC grade) were purchased from Sigma-Aldrich (Eschenstrasse 5, 82024, Taufkirchen, Germany).

Cheese sample

The cheese samples ($n = 3$) were a kind offer of the traditional cheese-making establishment owned by Mrs. Aggela Rouggeri (Creamery Tsiknias Mt., Steni Village, 84200, Tinos Island, Cyclades, Greece). The cheese was matured for three months before being delivered to the Department of Food Science and Nutrition (University of Thessaly, Terma N. Temponera, 43100, Karditsa, Greece). The preparation and production of the cheese was carried out in two stages. Firstly, the preparation of calabash, and secondly, the cheese production.

Calabash preparation

The process is relatively simple but tedious and laborious since calabash requires approximately three months for growth in the field to mature. After harvesting, the calabash was sun-dried for one year in order to lose as much moisture as possible to be converted into a container. After drying, it was cut around, and the inside was cleaned with boiling water.

Kariki cheese production

Kariki cheese was produced using cow milk (fat, protein, and lactose content was 3.83, 3.32, and 4.89%, respectively). In order to produce 1.2 kg of ready-made product, 20 kg of milk was needed. Once the fresh milk was collected, it was filtered to remove foreign matter. Then, the milk was pasteurised at 63 - 65°C for 30 min, and cooled until the temperature was lowered to 32°C. At this point, natural rennet (2 g per 100 L of milk) was added, and the pasteurised milk remained in the coagulation tank for 12 h.

The cheese curd was then cut with a special hand-held cube cutting tool, and placed on a stainless-steel bench for a short time, until it was transferred to special sacks that were hung on the cheese pan to drain for 24 h at ambient temperature. After this, the cheese curd was removed from the sacks, and placed in large sails on the cheese pan. The sails were then placed in the press for 24 h. After 24 h, and after the curd has turned into solid mass, it was grinded and salted (about 1% of NaCl). Then, it was kneaded by hand, and placed in the dried calabash. Finally, the calabash was sealed with a kind of natural glue (a mixture of flour and water) at the point where it was cut around, and placed in the maturing chamber for three months.

Physicochemical analyses of Kariki cheese

For physicochemical analyses, only the inner part of the cheese was used (after homogenisation). The moisture content of cheese sample was determined by the freeze-drying method using a Telstar Cryodos 80 freeze dryer (Telstar Industrial, S.A., Carrer de Josep Tapiolas, 120, 08226, Terrassa, Spain) for 24 h according to Lalas *et al.* (2011). The acidity

(expressed as lactic acid) was determined by titration according to James (1995). The protein determination was carried out using the Kjeldahl method (Ötles, 2011). The pH value of the cheese was determined electrometrically using a Hanna pH 210 meter (Hanna Instruments, Inc., Woonsocket, USA). The sodium chloride content was determined by Volhard method according to Haouet *et al.* (2006).

Fat extraction from Kariki cheese

The fat content was determined by means of Soxhlet apparatus according to James (1995). The fat from the dried cheese (about 5 g) was extracted with petroleum ether in a 250-mL Soxhlet apparatus, and analysed immediately. The fat content was determined after the evaporation of the solvent using a rotary evaporator (Laborota 4000, Heidolph, Walpersdorfer Str. 12, 91126, Schwabach, Germany).

Determination of fatty acid composition

The preparation of the fatty acid methyl esters (FAMEs) from cheese sample was carried out according to Commission Regulation (EC) No 796/2002 (EU, 2002). Approximately 0.1 g of the fat cheese sample were weighed in a 15-mL falcon tube. 2 mL of *n*-hexane were added, and mixed well. Then, 0.2 mL of 2 N methanolic potassium hydroxide solution was added, the mixture was vigorously vortexed for 30 s, and then left until the upper solution became clear. The upper layer containing the FAMEs was decanted, and then injected into GC.

The analysis of FAMEs was carried out with GC-FID according to a modified method described by Lalas *et al.* (2012). An Agilent Technologies (5301 Stevens Creek Blvd, Santa Clara, CA 95051 California, USA) Gas Chromatograph model 7890A, equipped with a capillary column Omegawax (30 m × 320 μm × 0.25 μm) (Supelco, 595 N Harrison Rd, PA 16823, Bellefonte, USA) was used. Helium was used as carrier gas at a flow rate of 1.4 mL/min. The oven temperature program was initially isotherm for 5 min at 70°C, then increased to 160°C with a heating rate of 20°C/min, then increased again to 200°C with a heating rate of 4°C/min, and finally up to 240°C with a rate of 5°C/min. The injector and flame ionisation detector (FID) temperatures were maintained at 240 and 250°C, respectively. For the FID detector, the flow rate for hydrogen was 50 mL/min, for air 450 mL/min, and the makeup flow 50 mL/min. Samples of 1.0 μL were injected into GC using the splitless mode. The individual peaks were identified by comparison to reference standards of Supelco 37-Component FAMEs Mix. The percentage composition of the samples was computed from the GC peak areas using the normalisation method (without correction factors). The component percentages were calculated as mean values from triplicate GC-FID analysis.

Determination of pigments

Pigment content was determined according to Minguez-Mosquera *et al.* (1991). Cheese fat (1.5 g) was dissolved in 5 mL of cyclohexane. The absorbance was measured at 470 nm (A_{470}) and 670 nm (A_{670}). Values of the extinction coefficients applied were E_o = 2,000 for lutein, and E_o = 613 for pheophytin-α. Pigment contents were calculated using Eqs. 1 and 2:

$$C_{\text{carotenoids}}\ (\text{mg/Kg of cheese}) = \frac{A_{470} \times 10^6}{2{,}000 \times 100 \times s} = A_{470} \times 5 \quad \text{(Eq. 1)}$$

$$C_{\text{chlorophylls}}\ (\text{mg/Kg of cheese}) = \frac{A_{670} \times 10^6}{613 \times 100 \times s} = A_{670} \times 16.3132 \quad \text{(Eq. 2)}$$

where, s = thickness of the quartz cells (in cm).

The carotenoid and chlorophyll concentrations were expressed as mg of lutein and pheophytin-α per kg of cheese, respectively. The total pigments ($C_{carotenoids} + C_{chlorophylls}$) and the ratio of chlorophylls/carotenoids were also calculated. Pigments present in the cheese samples were determined qualitatively by measuring the absorbance at 400 - 500 nm for carotenoids, and 550 - 710 nm for chlorophylls and their derivatives. The samples were scanned from 370 to 770 nm wavelength to get distribution of carotenoid and chlorophyll fractions in samples. A Shimadzu UV-1700 UV/Vis spectrophotometer (1-3 Kanda Nishiki-cho, Chiyoda-ku, 101-8448, Tokyo, Japan) was used for spectrophotometric measures. All samples were measured in triplicate.

Statistical analysis

Results were expressed as mean and standard deviation of three simultaneous assays (n = 3). Statistical significance of the differences between mean values was assessed by ANOVA; $p < 0.05$ was considered as statistically significant.

Results and discussion

Physicochemical analysis

The results of the physicochemical analyses are presented in Table 1. The 3-month matured Kariki cheese had 16.4% moisture content, 1.06% acidity (as lactic acid), and 4.2 pH. The total fat, protein, and sodium chloride contents were 44.8, 34.9, and 2.1%, respectively.

Table 1. Physicochemical characteristics of cheese.

Parameter	Mean
Moisture %	16.4 ±2.9
Acidity % (as lactic acid)	1.06 ± 0.01
pH	4.2 ± 0.00
Fat %	44.8 ± 0.00
NaCl %	2.1 ± 0.3
Protein %	34.9 ± 0.4

Values are mean ± standard deviation of triplicate determinations (n = 3).

FAMEs

The fatty acid (FA) composition of the Kariki cheese fat is presented in Table 2. The saturated fatty acids (SFAs) were 75.07% of the total FA. Palmitic acid was the predominant SFA (31.35%). The monounsaturated fatty acids (MUFAs) were 14.59%, with oleic acid being the predominant (10.96%). The total polyunsaturated fatty acids (PUFAs) were 2.04%, with linoleic acid being the predominant (0.76%). The ω-3 FAs were present at 0.85%, While at the ω-6 at 1.14%. According to the FAMEs analysis, Kariki showed significant higher SFAs, lower MUFAs and PUFAs, and higher ω-3 FAs than 52 Italian and French cheeses with different production methods and milk types analysed by Prandini *et al.* (2011). Additionally, Kariki's FAMEs profile was compared to that (as determined by Zlatanos, 2002) of other hard Greek cheeses (with similar maturing time), namely, Formaella, Graviera, Kefalotyri, Ladotyri, Opsimotyri. Kariki was proven to have higher total SFAs and lower total MUFAs and PUFAs.

Pigment analysis

The final colour of Kariki cheese (at the end of maturation) was derived from the pumpkin or the natural cultivation. The analysis indicated the presence of carotenoids (as lutein) in the cheese sample at 1.55 ± 0.03%, and chlorophylls (as pheophytin-α) at 2.78 ± 0.05%. In addition, the total pigments were 4.33 ± 0.08%, and the ratio chlorophylls/carotenoids was 1.79 ± 0.01%. Due to the lack of previous literature on Kariki cheese, it was impossible to make any comparison of the

Table 2. Results of fatty acids composition.

FAME		Percentage	FAMEs		Percentage
C4:0	Butyric	2.50 ± 0.11	C18:2ω-6c	Linoleic	0.76 ± 0.08
C6:0	Caproic	2.12 ± 0.04	C18:2ω-6t	Linolelaidic	0.05 ± 0.02
C8:0	Caprylic	1.36 ± 0.01	C18:3ω-6	γ-Linolenic	0.27 ± 0.01
C10:0	Capric	3.33 ± 0.03	C18:3ω-3	α-Linolenic	0.63 ± 0.02
C11:0	Undecanoic	0.26 ± 0.02	C20:0	Arachidic	0.16 ± 0.01
C12:0	Lauric	3.89 ± 0.04	C20:1ω-9	*cis*-11-Eicosenoic	0.12 ± 0.01
C13:0	Tridecanoic	0.18 ± 0.03	C20:2	*cis*-11,14-Eicosadienoic	0.03 ± 0.02
C14:0	Myristic	12.81 ± 0.07	C20:3ω-6	*cis*-8,11,14-Eicosatrienoic	0.08 ± 0.01
C14:1	Myristoleic	0.34 ± 0.03	C20:3ω-3	*cis*-11,14,17-Eicosatrienoic	0.17 ± 0.01
C15:0	Pentadecanoic	1.14 ± 0.02	C20:4ω-6	Arachidonic	0.03 ± 0.01
C15:1	*cis*-10-Pentadecenoic	0.27 ± 0.03	C21:0	Henicosanoic	0.15 ± 0.01
C16:0	Palmitic	31.35 ± 0.04	C22:0	Behenic	0.01 ± 0.00
C16:1	Palmitoleic	0.93 ± 0.02	C22:1ω-9	Erucic	0.02 ± 0.00
C17:0	Heptadecanoic	0.67 ± 0.02	C22:2	*cis*-13,16-Docosadienoic	0.02 ± 0.00
C17:1	*cis*-10-Heptadecenoic	0.27 ± 0.02	C22:6ω-3	*cis*-4,7,10,13,16,19-Docosahexaenoic	0.06 ± 0.01
C18:0	Stearic	17.80 ± 0.01	C23:0	Tricosanoic	0.02 ± 0.00
C18:1ω-9c	Oleic	10.96 ± 0.04	C24:0	Lignoceric	0.01 ± 0.00
C18:1ω-9t	Elaidic	1.94 ± 0.01	C24:1ω-9	Nervonic	0.02 ± 0.00

Values are mean ± standard deviation of triplicate determinations (n = 3).

Table 3. Cheeses with similar maturing time.

Type of cheese	Country of origin	Milk type[1]	Type of cultivation[2]	Maturing time (months)	pH	Water content (%)	Fat content (%)	Protein content (%)	NaCl (%)
Kariki	Greece	C	Natural rennet	3	4.2	16.4	44.8	34.9	2.1
Roquefort	France	S	LL, LC, PR	3	6.4	41.3	32.9	19.7	3.5
Danablu	Denmark	C	PR	2 - 3	5.3	45.9	30.0	20.4	3.1
Gorgonzola	Italy	C	ST, LB, PR	3 - 4	5.6	46.1	30.0	20.8	2.6
Tilsit	German	C	LL, ST, BLN	2 - 3	5.9	41.7	24.1	24.9	2.6
Cantal	France	C	LL, LC, ST	3	5.2	37.3	31.2	22.4	2.0
Cabrales	Spain	C + S + G	Natural rennet	2 - 5	5.3	38.5	36.0	22.0	2.6
Ladotyiri	Greece	S, S + G	ST, LB	3	5.3	33.8	35.6	24.7	2.0
Kefalograviera	Greece	S, S + G	ST, LB	3	5.2	35.9	34.5	24.9	2.7
Kasseri	Greece	S, S + G	ST, LB	3	6.0	43.1	27.9	24.1	1.3

[1]C = cow milk; G = goat milk; and S = sheep milk. [2]BLN = *Brevibacterium linens*; LB = *Lactobacillus delbrueckii* subsp. bulgaricus; LC = *Lactococcus lactis* subsp. cremoris; LL = *Lactococcus lactis* subsp. lactis; PR = *Penicillium roqueforti*; ST = *Streptococcus thermophiles*.

results obtained in the present work.

Comparison with similar cheeses

In Table 3, some cheeses (with similar maturing time) from different countries (Papademas and Bintsis, 2018) are listed in order to make characteristic comparison with those of Kariki cheese (Mantis *et al.,* 2015). These kinds of cheese were Roquefort (O'Brien and O'Connor, 2017), Danablu and Gorgonzola (Zarmpoutis *et al.,* 1997), Tilsit (Ozbekova and Kulmyrzaev, 2017), Cantal (Martin *et al.,* 2009), Cabrales (Cantor *et al.,* 2017), Ladotyri, Kefalograviera, and Kasseri (Andrikopoulos *et al.,* 2003). According to Fox and McSweeney (2017), the levels of moisture and salt, pH, and cheese microbiota regulate and control the biochemical changes that occur during ripening, hence determine the flavour, aroma, texture, and functionality of the finished product.

Kariki cheese presented a lower pH value and a significantly lower level of moisture as compared to other cheeses (Papademas and Bintsis, 2018). The low moisture and the high fat content were derived probably from the maturation process in the calabash. The calabash absorbed the cheese moisture and diffused it to the environment. Regarding pigments, no comparison can be made due to the lack of previous data. According to McSweeney (2017), fatty acids have a direct impact on the flavour of many cheese varieties, and particularly, C_4-C_{10} acids highly contribute to the flavour. As mentioned previously, Kariki had much higher SFAs, and lower MUFAs and PUFAs than the other (Greek or foreign) cheeses with similar maturing time (Zlatanos, 2002).

Conclusion

The present work aimed to identify, for the first time, the traditional manufacturing process and the physicochemical properties of the Kariki cheese originated from the island of Tinos, Greece. As indicated by the results, Kariki is a yellow hard cheese with low moisture, acidity, and salt content, and high proportion in protein and fat (thus, is highly nutritious). Kariki cheese is a rare delicatessen with a unique production procedure.

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