Changes of Free Fatty Acids during Ripening of Niva Cheese

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Abstract: Changes in the concentrations of free fatty acids in Niva cheese were monitored over the ripening period. Fatty acids were analyzed as methyl esters using gas chromatography with flame ionization detection. Identification has been carried out by comparison of the retention times with those of standard substances. Methanol esterification method using potassium hydroxide catalysis was used for preparing the sample of the fatty acids. The method is simple in respect to instrumentation and chemicals. It can be applied directly to the cheese matrix, which significantly decreases the time for sample preparation. There were total of 30 fatty acids identified in the cheese. Capric, myristic, palmitic, stearic and oleic acids represented the largest proportion of acid content and the most significant changes during ripening.

Keywords: blue cheese; fatty acids; gas chromatography

INTRODUCTION

Niva is a mould ripened cheese variety manufactured from pasteurized milk. The most important microorganism involved in the manufacture of blue cheeses is *Penicillium roqueforti* that is responsible for the unique characteristics of these varieties. For many years, manufacture of blue cheeses has been carried out in a completely natural way. However, nowadays the manufacture of these varieties under controlled conditions and the use of selected *Penicillium roqueforti* strains are current practices in the cheese industry and are necessary to obtain a product with the desired characteristics. Major attribute that influences the selection and consumption of cheeses is flavour formed during ripening of cheeses. The flavour of the blue cheeses is characterized by compounds derived from pronounced proteolysis and lipolysis due to the proteolytic and lipolytic activities of *Penicillium roqueforti*. Although extensive lipolysis may be considered undesirable in most cheese varieties, free fatty acids derived from milk fat contribute positively to the flavour of blue cheeses and they are precursors of more complex aroma compounds, such as methyl ketones, alcohols, lactones and esters. But at very high concentrations, fatty acids are perceived as off flavours. The aim of our work was to isolate, identify and quantify fatty acids of Niva cheese and monitor changes in their concentrations throughout ripening. Our results would provide important information contributing to knowledge of the biochemical processes involved in the ripening of Niva cheese.

EXPERIMENTAL

Cheeses. Three batches of Niva (blue mould cheese, 53% dry matter, 55% fat in dry matter) were manufactured following the traditional technology process in a selected dairy.

Cheeses were analyzed for fatty acids content at 1, 14, 27, 43 and 60 days of ripening. The samples were packaged in aluminum foil and stored at 5°C before use.

Methods. Fatty acids were analyzed as methyl esters using gas chromatography with flame ionization detection. Identification has been carried out by comparison of the retention times with those of standard substances.

The modified method of Šimek *et al*. [1] was used for methyl esters preparation. The cheese was finely ground and 5 g of a sample thus prepared was placed in a distillation flask with 50 ml methanolic 0.5 mol/l KOH. This mixture was esterified 30 min with water cooler. After cooling was mixture neutralized with concentrated H_2SO_4 and reesterified next 30 min. Methyl esters obtained were three times shaken in the separating funnel with 10 ml of heptane, the separated heptane layers were dried with anhydrous Na_2SO_4 and filtered to a 50 ml volumetric flask and filled up to the mark with heptane. This solution was transferred into the vial and 1 µl was injected by splitless injector (the linear purge was closed for 5 min) into the gas chromatographic column.

Fatty acids methyl esters were separated using gas chromatograph TRACE GC (ThermoQuest Italia S. p. A., I) equipped with flame ionization detector, split/splitless injector and capillary column SP™ 2560 (100 m × 0.25 mm × 0.2 µm) with the temperature programme 60°C held for 2 min, ramp 10°C/min up to 220°C, held for 20 min. The injector temperature was 250°C and the detector temperature was 220°C. The flow rate of the carrier gas N_2 was 1.2 ml/min.

RESULTS AND DISCUSSION

A mixture of 37 methyl ester standards of fatty acids Supelco 37 Component FAME mix (Supelco, Sigma-Aldrich, USA) was used for qualitative evaluation. The following fatty acids were determined in Niva cheese: caproic (C6:0), caprylic (C8:0), capric (C10:0), undecanoic (C11:0), lauric (C12:0), tridecanoic (C13:0), myristic (C14:0), myristooleic (C14:1 n9c), pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0), heptadecenoic (C17:1), stearic (C18:0), elaidic (C18:1 n9t), oleic (C18:1 n9c), linolelaidic (C18:2n6t), linoleic (C18:2n6c), arachidic (C20:0), γ-linolenic (C18:3n6), eicosenoic (C20:1), linolenic (C18:3), heneicosanoic (C21:0), eicosadienoic (C20:2), behenic (C22:0), eicosatrienoic (C20:3n6), erucic (C22:1n9), eicosatrienoic (C20:3n3), arachidonic (C20:4n6).

The method showed good reproducibility (RSD in range 3–5%), linearity was good for the selected fatty acids in the concentration range 0.05–50 µg/g of cheese and the limits of detection differred slightly for the various fatty acids (they were in range 0.05–0.5 µg/g of cheese).

It is widely accepted that several free fatty acids contribute directly to the final flavour characteristics of many types of cheese or indirectly by serving as precursors of some aroma components. Above all alkanoic acids with carbon chains from C2 to C10 seem to have a major effect on the flavour of cheeses [2]. For example Partidário *et al*. [3] reported, that free fatty acids are associated with the characteristic flavour of ewe cheese. Tuomala and Kallio [4] found, that volatile fatty acids are the major contributors to Swiss cheese flavour.

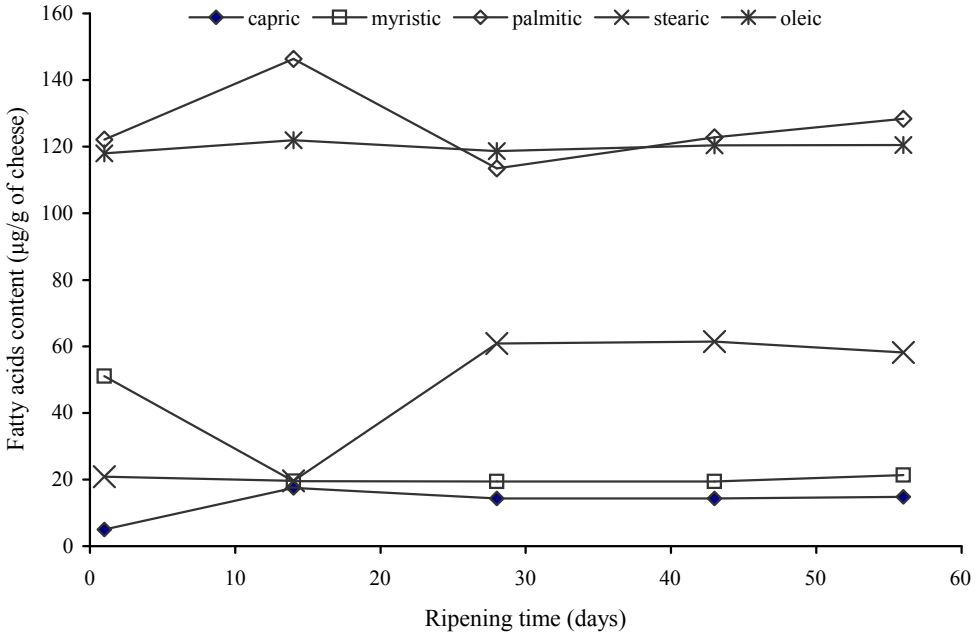

Figure 1. Changes of fatty acids methyl esters content during ripening of Niva cheese

Table 1. Changes of fatty acids methyl esters content during ripening of Niva cheese (μg/g of cheese)

Fatty acid	Ripening time (days)				
	1	14	27	43	60
Caproic	9.49 ± 0.39	8.43 ± 0.37	6.32 ± 0.29	7.20 ± 0.33	7.29 ± 0.11
Caprylic	7.47 ± 0.44	6.83 ± 0.41	5.25 ± 0.34	5.63 ± 0.37	6.19 ± 0.12
Capric	4.95 ± 0.52	17.46 ± 0.58	14.36 ± 0.07	14.29 ± 0.11	14.83 ± 0.02
Undecanoic	2.85 ± 0.04	2.68 ± 0.16	2.54 ± 0.28	2.34 ± 0.02	2.38 ± 0.04
Lauric	18.57 ± 0.46	19.39 ± 0.10	17.84 ± 0.88	16.02 ± 0.51	17.93 ± 0.23
Tridecanoic	1.32 ± 0.23	1.21 ± 0.02	1.42 ± 0.05	1.29 ± 0.06	1.33 ± 0.11
Myristic	51.06 ± 1.74	19.46 ± 0.53	19.45 ± 0.17	19.39 ± 1.18	21.33 ± 0.73
Myristooleic	2.13 ± 0.95	2.12 ± 0.12	5.26 ± 0.12	3.18 ± 0.45	2.60 ± 0.68
Pentadecanoic	5.47 ± 2.80	6.99 ± 0.29	12.71 ± 0.11	7.19 ± 0.56	5.23 ± 1.20
Palmitic	122.18 ± 0.36	146.37 ± 0.22	113.47 ± 1.72	122.78 ± 1.39	128.35 ± 3.03
Heptadecanoic	–	–	12.04 ± 5.61	4.98 ± 0.39	3.21 ± 1.60
Heptadecenoic	–	–	–	–	0.23 ± 0.11
Stearic	20.84 ± 2.32	19.57 ± 0.45	60.87 ± 0.72	61.36 ± 0.95	58.12 ± 2.79
Elaidic	0.71 ± 0.36	–	–	–	–
Oleic	118.03 ± 2.07	121.97 ± 0.79	118.69 ± 2.37	120.38 ± 1.12	120.53 ± 4.94
Linolelaidic	0.07 ± 0.01	0.33 ± 0.17	0.36 ± 0.00	0.12 ± 0.04	0.39 ± 0.28
Linoleic	1.40 ± 0.23	1.73 ± 0.03	1.87 ± 0.11	1.25 ± 0.04	1.52 ± 0.01
Arachidic	30.60 ± 0.35	34.23 ± 0.41	28.08 ± 0.47	29.95 ± 0.56	-
γ-linolenic	0.21 ± 0.01	0.68 ± 0.47	0.15 ± 0.01	0.20 ± 0.01	0.20 ± 0.02
Eicosenoic	0.05 ± 0.03	0.08 ± 0.04	0.23 ± 0.12	0.26 ± 0.01	0.32 ± 0.01
Linolenic	–	–	0.24 ± 0.00	–	0.40 ± 0.01
Heneicosanoic	–	–	–	0.21 ± 0.11	3.46 ± 0.16
Eicosadienoic	0.13 ± 0.02	0.47 ± 0.00	0.28 ± 0.05	0.69 ± 0.05	0.71 ± 0.08
Behenic	–	–	–	0.36 ± 0.08	0.59 ± 0.29
Eicosatrienoic n6	0.51 ± 0.02	0.14 ± 0.00	0.32 ± 0.03	0.27 ± 0.02	0.78 ± 0.39
Erucic	0.54 ± 0.27	–	–	–	0.00 ± 0.00
Eicosatrienoic n3	0.36 ± 0.09	0.56 ± 0.00	0.34 ± 0.00	0.41 ± 0.02	0.57 ± 0.01
Arachidonic	0.92 ± 0.01	1.16 ± 0.02	1.02 ± 0.03	0.88 ± 0.04	–

However, the relation between total free fatty acid composition and final cheese flavour is still not completely established. Although the appearance of free fatty acids in such a complex system as cheese should in general be considered as the net result of several possible processes (e.g. enzymatic hydrolysis of existing glycerides, biosynthesis effected by the microflora and nonenzymatic oxidation of long and/or unsaturated fatty acids), the hydrolytic action of lipases is probably the main route for the formation of free fatty acids especially because of the normal pH range of cheeses. Lipases in cheese may originate from the milk, from the indigenous (or added) microorganisms, and possibly from rennet [5].

Changes in the fatty acids contents during ripening of Niva cheese are presented in Table 1 and in Figure 1. As can be seen, the concentration of each fatty acid slightly changed, capric, myristic, palmitic, stearic and oleic acids represented the largest proportion of acid content and the most significant changes during ripening. This lack of changes can be caused by the equilibrium between creation of acids from milk fat and their decomposition to another aroma compounds. Nevertheless, concentrations of fatty acids with carbon chains to C10 were considerably higher than their flavour thresholds found in references [2] and therefore they can influence flavour of Niva cheese. Fatty acids with carbon chain higher than C10 have

high flavour thresholds and they probably did not contribute to flavour of Niva cheese.

CONCLUSIONS

Methanol esterification method using potassium hydroxide catalysis was used for preparing the sample of the fatty acids. The method is simple in respect to instrumentation and chemicals. It can be applied directly to the cheese matrix, which significantly decreases the time for sample preparation.

There were total of 30 fatty acids identified in Niva cheese. Capric, myristic, palmitic, stearic and oleic acids represented the largest proportion of acid content and the most significant changes during ripening of cheese.

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