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Formation of sensory active substances during ripening of Dutch-type cheese with reduced salt content

Irena Němečková[1], Šárka Trešlová[1], Helena Čížková[2], Tereza Rambousková[2], Jan Forejt[1], Zdeněk Švandrlík[1], Vojtěch Kružík[2], Dana Gabrovská[3]*

[1]*Dairy Research Institute, Prague, Czech Republic*
[2]*Department of Food Preservation, Faculty of Food and Biochemical Technology, University of Chemistry and Technology, Prague, Czech Republic*
[3]*Czech Institute for Agri-Food Research and Innovation, Prague, Czech Republic*

**Corresponding author: nemeckova@milcom-as.cz*

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Abstract: The reduction of NaCl content in cheeses is nutritionally desirable but quite challenging due to NaCl's key role during cheese production and ripening. We focused on reformulated Dutch-type cheeses ripened for 120 days and their microbiological and sensory characteristics, including of determining organic acids (electrophoresis) and volatile substances (SPME-GC-MS analysis). Experimental batches contained 0.64, 0.90, and 1.19% NaCl or 0.77% NaCl together with 0.33% KCl. The influence of salts on lactose and citrate metabolism (the formation of lactic, acetic, and formic acid, ethanol, diacetyl, acetoin and 2,3-butanediol), proteolysis (the formation of glutamic acid), lipolysis and β-oxidation of fatty acids (the formation of 2-butanone, 2-butanol, hexanal, hexanoic and octanoic acid) was undetected. Contrarily, brining conditions affected the contamination of cheese surfaces with yeasts and halotolerant microorganisms and cheese consistency. While a typical consistency was formed only in the cheeses with 1.19% NaCl acceptable saltiness was declared in the cheeses with the content of salts 0.90% or higher. The partial replacement of NaCl with KCl caused metallic off-taste in the cheeses that ripened longer than Consistent acceptance seems to be the most limiting factor for the tested reformulation appears.

Keywords: brining; cheese microbiome; organic acids; reformulation; volatile substances

Excessive intake of sodium is one of the main health risks of the western diet. To reduce blood pressure and the risk of cardiovascular disease, stroke and coronary heart disease in adults, World Health Organization (WHO) recommends reducing the daily intake of sodium to less than 2 g (less than 5 g salt daily) while the molar ratio of sodium to potassium should be approximately one to one (WHO 2012). Nevertheless, this authority admits that most people do not follow the recommendation and consume too much salt

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(in an average of 9–12 g daily) and not enough potassium (less than 3.5 g daily). In many countries, most salt comes from processed foods (e.g. ready meals, snacks, processed meat products, cheeses), from foods consumed frequently in large amounts (bread), or salt is added during cooking or at the point of consumption (WHO 2018). This work is focused on the reduction of salt content in Dutch-type cheeses.

In general, the decision to simply reduce salt content in cheeses by itself is not competent because NaCl affects not only the nutritional parameters but also sensory characteristics, technological and microbiological parameters, shelf-life, and safety of cheeses. Sodium chloride plays a key role during cheese production and ripening. It affects various parameters, processes, and interactions, e.g. water activity, the activity of rennet and other enzymes, the growth and activity of starter and non-starter lactic acid bacteria (NSLAB), spoilage microorganisms and pathogens, as well as their interactions, whey drainage, biochemical changes during ripening, consistency changes and the formation of sensory active substances (Kloss et al. 2015).

Besides the simple reduction of salt content, reformulated cheeses can contain salt replacers. For example, KCl is supposed to exert similar effects and technological functions as NaCl. However, complete replacement is seldom feasible due to metallic and bitter off-tastes (Kloss et al. 2015, Costa et al. 2018).

Moreover, above-mentioned characteristics can vary among different types of reformulated cheeses. Some authors (Juan et al. 2022; Tidona et al. 2022) have discussed various semi-hard kinds of cheese and focused only on basic physicochemical, microbiological, and sensory characteristics. More specific data are available, e.g. on semi-hard Cheddar-cheeses. Møller et al. (2013) or Murtaza et al. (2014) focused on proteolysis and the formation of bitter peptides as well as on textural changes. Murtaza et al. (2014) also involved the profiles of volatile flavour compounds. In exact reference to semi-hard Dutch-type cheeses, only a restricted dataset is available. Czarnacka-Szymani and Jezewska-Zychowicz (2015) studied consumers' preferences and found salt content reduction by about 15% acceptable, despite differences in sensory and textural profiles. Hoffmann et al. (2020) focused on Edam cheese ripened for six weeks and its microbiological and sensory characteristics, as well as on proteolysis and the formation of volatile compounds. They reduced salt content below 1% and also tested a partial NaCl replacement with KCl. However, the reformulated cheeses were unacceptable due to their bitter taste. We aimed to contribute to a better understanding of complex processes during the ripening of semi-hard Dutch-type cheese with reduced NaCl content and with or without the use of KCl as a partial replacer. Special attention was paid to the formation of organic acids and volatile compounds as less frequently studied parameters of reformulated cheeses. Our findings were given into the context with other characteristics of cheeses: basic physical and chemical parameters (general conditions), changes in the microbiome (the producers of sensory active substances) and sensory acceptance (the reformulation as manifested to consumers).

MATERIAL AND METHODS

Cheese production. During one production day, pasteurised bactofuged cow milk directly from a cheese-production plant in a volume of 250 L was used. Calcium chloride (103.75 mL of saturated solution) and liquid mesophilic starter CCDM 1 (MILCOM, Czech Republic) in a volume of 1.25 L were added. After one-hour incubation at 32–33 °C, rennet CHY-MAX M 1000 (Chr. Hansen, Germany) in a volume of 10.5 mL was added as well. Total time from starter addition to whey drainage took 120 min and 97.5 L of whey was obtained. Afterwards, 57.5 L of cooking water heated to 55 °C was added. Scalding took time for 25 min at 37–38 °C. Subsequent steps were moulding for 20 min, pressing for one hour and acidification to pH 5.2. This procedure yielded 20 one-kilogram-loafs of cheese. Afterwards, brining proceeded at 14 °C in a real 20% cheese-production brine or in a laboratory brine with quarter molar NaCl replacement consisting of 812 g KCl, 1 890 g NaCl, 195 mL lactic acid, 50 g $CaCl_2$ and 12 L H_2O (pH adjusted to 5.2 ± 0.2). Five-loaf batches were brined for 4 h (batch B1), 8 h (batch B2) and 16 h (batch B4) in the real brine or 8 h (batch B3) in the laboratory brine. Then, the cheeses were wiped, packed in a shrinkable foil, and ripened at 15 °C. One loaf from each batch was sampled after 7, 30, 60, 90, and 120 days of ripening, respectively. For analyses, samples were prepared as loaf sectors grated and homogenised either as a whole or as a one-centimetre surface layer and a core. The whole experiment was carried out two times, with the beginning of cheese production in the course of two weeks.

Physical and chemical analyses. Basic physical and chemical parameters were determined using relevant standards: active acidity and dry matter accord-

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ing to ISO3433 (ISO 3433: Methods of test for cheese, curds, creams and spreads. Geneva, International Organization for Standardization, 2008) and water activity according to ISO 18787 (ISO 18787: Foodstuffs – Determination of water activity. Geneva, International Organization for Standardization, 2017). NaCl content was determined using atomic absorption spectrometry according to EN 1134 [EN 1134: Fruit and vegetable juices. Determination of sodium, potassium, calcium and magnesium content by atomic absorption spectrometry (AAS). Brussels, European Committee for Standardization, 1993] and total chlorides by Mohr's argentometric titration according to ISO 9297 [ISO 9297: Water quality. Determination of chloride. Silver nitrate titration with chromate indicator (Mohr's method). Geneva, Switzerland, International Organization for Standardization, 1989].

Organic acids were determined by an automatic electrophoretic analyser EA 02 (VILLA Labeco, Slovak Republic) using its intrinsic protocol. Volatile compounds were determined using a solid phase microextraction followed by gas chromatography with mass spectrometry (SPME-GC-MS) analysis, namely Supelco SPME fibre 50/30 μm 24 Ga divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Merck, Germany), GC-Autosampler (CTC Analytics, Switzerland), gas chromatograph 7890 B with a colon HP-5MS and mass triple-axis detector 5977 A (Agilent, USA). A one-gram sample was placed into a 10 mL SPME vial, and an internal standard (10 μL of aqueous 2-methyl-3-heptanone solution, 16.2 $mg \cdot L^{-1}$) was added. The chromatographic conditions were according to the methodology described earlier by Hanková et al. (2020). Particular volatile compounds were identified by spectrum comparison with NIST 14 mass spectra library and using retention indexes measured for identified compounds and C5–C8 and C18–C20 alkanes as standards. Peak areas were used for the quantification.

Microbiological analyses. Microbiological parameters were determined by plating and colony counting under conditions recommended by the producer of cultivation media specialised in dairy analyses (MILCOM, Czech Republic). Mesophilic lactic cocci were cultivated on M17 agar at 30 °C for 3 days, heterofermentative *Lactobacillaceae* on FHN + VN agar at 30 °C for 6 days anaerobically, yeasts and moulds on GKCH agar at 25 °C for 5 days, coliforms, and *E. coli* on chromogenic CHR agar at 37 °C for 24 h and halotolerant microorganisms on MS agar at 30 °C for 3 days. The composition of cultivation media is specified in Table 1.

Sensory evaluation. A panel of ten trained assessors from our cheese laboratory was asked to identify sensory defects of samples (free characteristics), assess saltiness adequacy (too low/sufficient/too high), and select the most preferred sample.

Statistical analyses. The contents of salt, dry matter and volatile compounds were determined in whole cheese sectors. In contrast, the content of chlorides and organic acids, microbiological parameters, water activity and acidity in surface layers and cores were separate. Unless mentioned otherwise, results were obtained in duplicates from two production series (total $n = 4$) and expressed as arithmetic means with standard deviations. For better clarity, only selected results are shown. The analysis of variance was not involved because the natural variability of microbiological parameters, organic acids and volatile compounds in the parallel production experiments and individual samples was about several-fold more pronounced than the differences among the batches with various salt content. It means that found statistically significant differences in the dataset would be distributed randomly.

Table 1. Composition of cultivation media

MILCOM medium	Composition
M17	The same as M17 Agar acc. to Terzaghi, Merck, Germany.
FHN + VN	Protease peptone 10.0 g; meat extract 10.0 g; yeast extract 1.0 g; D-mannitol 20.0 g; $MnSO_4 \cdot 4\ H_2O$·0.1 g; $MgSO_4 \cdot 7\ H_2O$·0.1 g; Tween 80 1.0 mL; acetate buffer 1 $mol \cdot L^{-1}$ pH 5.4 200 mL; agar 9.0–18.0 g and demineralised water 1 L. After sterilisation, supplement with vancomycin to 50 $mg \cdot L^{-1}$ and with nalidixic acid to 40 $mg \cdot L^{-1}$. Final pH 5.4 ± 0.2.
GKCH	The same as Yeast Extract Glucose Chloramphenicol Agar FIL-IDF, Merck, Germany.
CHR	The same as Chromocult Coliform Agar, Merck, Germany.
MS	The same as Mannitol Salt Phenol-Red Agar, Merck, Germany.

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Table 2. Basic characteristics of cheese batches B1–B4 (n = 20, whole cheese sectors)

Batch	Brining time (h)	Salt type	NaCl (%)	Dry matter (%)
B1	4	NaCl	0.64 ± 0.03	56.72 ± 1.73
B2	8	NaCl	0.90 ± 0.04	57.18 ± 1.98
B3	8	NaCl and KCl	*0.77 ± 0.04	56.82 ± 1.55
B4	16	NaCl	1.19 ± 0.06	57.07 ± 1.91

* the sum of NaCl and KCl can be estimated at 1.10%

RESULTS AND DISCUSSION

Physical and chemical parameters. Four batches of cheese were produced. Their basic characteristics are summarised in Table 2. The content of NaCl and dry matter were stable during ripening. Thus, results are expressed as arithmetic means from data measured in all tested ripening times. The results on NaCl content represent the nutritional values of produced cheeses.

Chosen physical and chemical parameters logically linked together are shown in Table 3. Compared to batches B1–B4, the higher content of chlorides (NaCl, KCl, and $CaCl_2$) the lower the water activity, e.g. after 30-day ripening, the linear regression coefficient was 0.9978. Contrary, pH and glutamic acid content were unaffected by chlorides.

Compared to the surface and core of cheese, the diffusion of chlorides can be observed. Chlorides diffused from the surface to the core, especially during the first month of ripening. In more ripened cheeses, the surface and core did not differ in any physical or chemical parameter determined in this work. Principally, our

Table 3. Physical and chemical parameters of cheese batches B1–B4 (n = 4, cheese surfaces and cores)

Parameter	Ripening time (days)	Sample type	B1	B2	B3	B4
Chlorides (%)	7	surface	0.92 ± 0.02	1.29 ± 0.00	1.46 ± 0.08	1.70 ± 0.03
		core	0.55 ± 0.06	0.65 ± 0.06	0.80 ± 0.16	0.86 ± 0.15
	30	surface	0.83 ± 0.06	1.22 ± 0.11	1.33 ± 0.06	1.51 ± 0.07
		core	0.79 ± 0.04	1.16 ± 0.06	1.37 ± 0.03	1.46 ± 0.08
	120	core	0.86 ± 0.08	1.17 ± 0.08	1.35 ± 0.09	1.56 ± 0.17
Water activity	7	surface	0.976 ± 0.000	0.976 ± 0.008	0.968 ± 0.002	0.965 ± 0.000
		core	0.982 ± 0.000	0.982 ± 0.000	0.977 ± 0.000	0.978 ± 0.003
	30	surface	0.973 ± 0.000	0.969 ± 0.001	0.968 ± 0.003	0.962 ± 0.001
		core	0.976 ± 0.002	0.970 ± 0.003	0.966 ± 0.003	0.965 ± 0.001
	120	core	0.967 ± 0.004	0.964 ± 0.003	0.961 ± 0.005	0.957 ± 0.006
Active acidity	7	surface	5.42 ± 0.07	5.37 ± 0.05	5.41 ± 0.02	5.36 ± 0.01
		core	5.41 ± 0.00	5.40 ± 0.02	5.41 ± 0.03	5.39 ± 0.01
	30	surface	5.51 ± 0.10	5.46 ± 0.09	5.46 ± 0.08	5.48 ± 0.08
		core	5.47 ± 0.06	5.46 ± 0.07	5.53 ± 0.07	5.43 ± 0.03
	120	surface	5.66 ± 0.05	5.65 ± 0.09	5.67 ± 0.05	5.61 ± 0.17
Glutamic acid ($mg \cdot kg^{-1}$)	7	surface	545 ± 21	495 ± 120	545 ± 49	535 ± 35
		core	595 ± 92	565 ± 495	510 ± 42	510 ± 141
	30	surface	1 400 ± 452	1 335 ± 360	1 485 ± 728	1 415 ± 445
		core	1 415 ± 728	1 430 ± 763	1 435 ± 502	1 415 ± 700
	120	core	4 565 ± 1 888	5 185 ± 1 321	4 795 ± 1 647	5 030 ± 1 626

B1–B4 – mean values ± standard deviation (SD)

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Table 4. Organic acids as fermentation products in cheese batches B1–B4 determined using electrophoresis (n = 4, cheese cores)

Compound	Ripening time (days)	B1	B2	B3	B4
Lactic acid ($mg \cdot kg^{-1}$)	7	13 055 ± 1 580	13 366 ± 1 464	13 627 ± 1 612	13 655 ± 1 732
	120	13 298 ± 1 142	13 521 ± 1 500	12 945 ± 1 238	13 340 ± 2 436
Formic acid ($mg \cdot kg^{-1}$)	7	182 ± 22	167 ± 21	170 ± 22	163 ± 21
	120	190 ± 12	179 ± 17	199 ± 12	184 ± 9
Acetic acid ($mg \cdot kg^{-1}$)	7	1 325 ± 83	1 294 ± 44	1 326 ± 117	1 308 ± 76
	120	1 428 ± 99	1 490 ± 13	1 382 ± 128	1 458 ± 104

B1–B4 – mean values ± standard deviation (SD)

results are in accordance with data on more punctually analysed layers of Dutch-type cheese by Pachlová et al. (2011).

Compared to less and more ripened cheese, water activity reflected both the diffusion of chlorides and the production of low-molecular substances. Proteolysis, as the major ripening process, can be indicated by the increasing content of deliberated glutamic acid or by the increase in pH connected with amino acid catabolism. This process can lead to the formation of carbonyl compounds, organic acids, ammoniac or sulphuric compounds and biogenic amines (McSweeney 2011). NaCl in concentrations of about 1–2% supported the production of biogenic amines by NSLAB, particularly *Limosilactobacillus reuteri* (Body et al. 2021).

Besides glutamic acid, organic acids determined using electrophoresis are connected with fermentation processes (Table 4). These data are complemented with results on volatile compounds determined using SPME-GC-MS (Table 5). At the same time, lactic acid

Table 5. Volatile compound in cheese batches B1–B4 expressed as SPME-GC-MS peak areas ($\times 10^4$, abundance × second, n = 4, cheese sectors)

Compound	Aroma precepts*	Ripening time (days)	B1	B2	B3	B4
Ethanol	sweet, alcohol	7	109 ± 54	115 ± 17	90 ± 21	89 ± 35
		120	101 ± 41	109 ± 45	103 ± 46	98 ± 36
Diacetyl	butter	7	106 ± 32	106 ± 35	64 ± 25	119 ± 37
		120	38 ± 32	49 ± 36	48 ± 35	31 ± 12
2-butanone	lactic, ether	7	< 1	< 1	< 1	< 1
		120	250 ± 245	229 ± 235	304 ± 303	284 ± 268
2-butanol	medicine, fruit	7	< 1	< 1	< 1	< 1
		120	81 ± 57	48 ± 36	41 ± 30	37 ± 18
Acetoin	butter, cream	7	712 ± 496	1 008 ± 835	706 ± 646	682 ± 333
		120	289 ± 201	363 ± 265	444 ± 292	390 ± 261
Hexanal	grass, tallow, fat	7	13 ± 5	12 ± 3	10 ± 4	10 ± 2
		120	< 1	< 1	< 1	< 1
Butyric acid	rancid, cheese, sweat	7	< 1	< 1	< 1	< 1
		120	34 ± 7	28 ± 10	39 ± 5	32 ± 11
2,3-butanediol	fruit, onion	7	185 ± 44	124 ± 74	94 ± 36	80 ± 28
		120	287 ± 110	221 ± 44	291 ± 65	267 ± 90
Hexanoic acid	cheese, sweat	7	7 ± 5	4 ± 3	3 ± 2	3 ± 1
		120	11± 2	11± 2	7± 1	8± 2
Octanoic acid	sweat, cheese	7	5 ± 3	3 ± 2	2 ± 1	2 ± 1
		120	5 ± 2	4 ± 1	4 ± 1	4 ± 1

* https://flavornet.org/flavornet.html; B1–B4 – mean values ± standard deviation (SD)

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was formed from lactose by all species present in the starter (*Lactococcus lactis* ssp. *lactis*, *Lcc. lactis* ssp. *cremoris*, *Lcc. lactis* ssp. *lactis* biovar *diacetylactis*, *Leuconostoc* spp.) aroma-producers (*Lcc. lactis* ssp. *lactis* biovar *diacetylactis*, *Leuconostoc* spp.) co-metabolised lactose and citrate to form lactic, acetic, and formic acid, ethanol, diacetyl, acetoin and 2,3-butanediol (McSweeney 2011). While lactic and acetic acid, ethanol, diacetyl, and acetoin were present already from cheese production, the concentration of diacetyl and acetoin decreased to form 2,3-butanediol during ripening. Moreover, lactic acid can be partially oxidised to acetic acid during ripening (McSweeney 2011).

Using electrophoresis, the concentrations of undesirable propionic and butyric acid were below the detection limit (less than 1 mg·kg^{-1}). Using SPME-GC-MS, small amounts of butyric acid were detected after 120-day ripening due to the higher sensitivity of this method. Nevertheless, late blowing in cheeses is connected with the concentration of butyric acid as high as about 100–1 000 mg·kg^{-1} (Kavková et al. 2018).

The profile of volatile compounds included substances connected with microbial lipolysis and further β-oxidation of fatty acids. An increase in the concentration of 2-butanone and 2-butanol during ripening was the most pronounced. Nevertheless, small amounts of hexanal, hexanoic, and octanoic acid were also detected. Although lipolysis in Dutch-type cheeses is limited, short-chain organic acids and derived ketones, esters, thioesters, lactones, aldehydes, or alcohols in low concentrations are the typical components of cheesy flavour (McSweeney 2011). E.g. in Gouda cheese, Jung et al. (2013) identified 31 flavour compounds, including hexanal, hexanoic and octanoic acid but neither 2-butanone nor 2-butanol.

Ten volatile compounds shown in Table 5 were detected at least in one sample in a relative ratio higher than 0.1%. Totally, 31 volatile compounds were quantified, and any systematic influence of salt content was not observed.

Microbiological parameters. Moulds, coliforms, and *E. coli* in all samples were below the detection limit (less than 1 log CFU·g^{-1}, CFU – colony forming unit). The other determined microbiological parameters are shown in Table 6. The density of mesophilic lactic cocci and heterofermentative *Lactobacilaceae* did not depend on NaCl content or brining conditions. Moreover, mesophilic lactic cocci were evenly distributed in the cheeses, and their density during 120-day ripening decreased by about one decimal order. On the contrary, heterofermentative *Lactobacillaceae* were distributed unevenly in cheeses and among cheeses, especially, at the beginning of ripening. Nevertheless, these differences were subdued during ripening while

Table 6. Microbiological parameters of cheese batches B1–B4 (n = 4, cheese surfaces and cores)

Parameter	Ripening time (days)	Sample type	B1	B2	B3	B4
Mesophilic lactic cocci (log CFU·g^{-1})	7	surface	8.51 ± 0.44	8.33 ± 0.76	8.45 ± 0.47	8.26 ± 0.31
		core	8.69 ± 0.38	8.70 ± 0.39	8.61 ± 0.06	8.46 ± 0.36
	120	surface	7.66 ± 0.98	7.36 ± 0.90	7.28 ± 0.96	6.74 ± 0.65
		core	6.99 ± 1.40	6.89 ± 1.26	6.73 ± 1.35	6.74 ± 1.23
Heterofermentative *Lactobacillaceae* (log CFU·g^{-1})	7	surface	2.45 ± 2.05	3.07 ± 2.93	2.78 ± 2.52	2.81 ± 2.56
		core	1.52 ± 0.74	ND	3.02 ± 2.86	2.78 ± 2.51
	120	surface	6.89 ± 0.80	6.78 ± 1.09	6.71 ± 1.00	6.07 ± 1.18
		core	6.73 ± 1.49	6.65 ± 1.07	6.98 ± 0.64	6.60 ± 1.12
Yeasts (log CFU·g^{-1})	7	surface	3.25 ± 0.50	3.68 ± 0.02	ND	3.26 ± 0.26
		core	ND	ND	ND	ND
	120	surface	4.80 ± 0.48	4.31 ± 0.28	3.08 ± 1.20	4.00 ± 0.20
		core	2.56 ± 0.73	2.47 ±0.13	ND	2.21 ± 0.38
Halotolerant microorganisms (log CFU·g^{-1})	7	surface	2.35 ± 1.25	2.07 ± 1.52	1.57 ± 0.81	1.45 ± 0.63
		core	1.34 ± 0.48	1.47 ± 0.01	1.42 ± 0.60	1.14 ± 0.20
	120	surface	4.24 ± 0.28	4.82 ± 0.13	2.37 ± 1.51	4.75 ± 0.78
		core	2.20 ± 0.62	3.33 ± 0.75	1.13 ± 0.18	3.93 ± 0.16

ND – not detected; CFU – colony forming unit; B1–B4 – mean values ± standard deviation (SD)

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the density of heterofermentative *Lactobacillaceae* increased, on average, about four decimal orders.

On the other hand, the density of yeasts and halotolerant microorganisms depended on NaCl content and brining conditions and differed between the surface and core of cheeses during the whole ripening period. The real brine was a good source of yeasts and halotolerant microorganisms contaminating mainly cheese surfaces. Especially, the density of halotolerant microorganisms in the cheese surfaces increased with prolonged brining time. Contrary, cheeses brined in the laboratory brine were contaminated the least. Both differences among batches and individual samples led to a variable increase in the density of yeasts and halotolerant microorganisms during ripening which was about one to three decimal orders.

Sensory evaluation. All cheeses' saltiness except batch B1 (0.64% NaCl) was sufficient. Cheeses were acceptably ripened after 60 days when batch B4 was the most preferred. However, in more ripened cheeses (90 and 120 days of ripening) with a more complex sensory profile, batches B2 and B3 with slightly reduced NaCl content were preferred.

The sensory defects and off-tastes of cheeses, as they appeared during ripening, are indicated in Table 7. In one of the production experiments, surface rusty spots appeared after 120-day ripening in batches brined in the real brine. Yeast originating in the brine are supposed to be the cause. Nevertheless, any other microbial defect of appearance or consistency was not observed. However, the formation of proper consistency was conditioned by a reasonable salt content as in batch B4 (1.19% NaCl). The untypical consistency of less salted cheeses was the most serious observed defect. Similarly, in Cheddar cheeses, desirable hardness, toughness, and crumbliness decreased with the reduction of salt content (Murtaza et al. 2014).

Attention was also paid to the formation of sensory active substances and relevant off-tastes during ripening. In the retail market in the Czech Republic, most Dutch-type cheeses are ripened for 60 days or shorter. During this period, fewer salted cheeses (batches B1, B2, and B3) were insipid. Furthermore, all cheeses (batches B1, B2, B3, and B4, respectively) after 60-day ripening were slightly bitter as caused by short hydrophobic peptides. Hoffmann et al. (2020) indicated the bitterness of Edam cheeses with various NaCl replacers after six-week ripening as unacceptable. Nevertheless, in our work, bitter peptides were broken down later, and the bitterness of cheeses disappeared. In this process, intracellular peptidases released from lysed starter bacteria play a key role (McSweeney 2011). Reducing bitterness is desirable, especially in cheeses with reduced salt content. E.g. in Gouda cheeses with less intensive bitterness, saltiness appeared more intensive (Němcová et al. 2001). Completing the dataset using a texture profile analysis or study on proteolysis (e.g. peptide profiles or the determination of free amino groups) could bring further interesting results. In cheeses ripened for 90 days or longer, proteolysis proceeded amino acids (which can be connected with a sweetish taste), and lipolysis became pronounced.

Table 7. Sensory defects and off-tastes of cheese batches B1–B4 (n = 20, cheese sectors)

Defect	Ripening time (days)	B1	B2	B3	B4
In appearance	120	surface rusty spots	surface rusty spots	–	surface rusty spots
In consistency	60	soft, smeary, mealy	smeary surface, granulated core	gummy	–
In aroma	–	–	–	–	–
In taste	30	–	slightly bitter	insipid	slightly bitter
	60	slightly bitter, insipid	insipid	slightly bitter	–
	90	sweetish	astringent, hot, sweetish	–	rancid, hot
	120	astringent	–	rancid, metallic, sweetish	sweetish

Ripening time indicates when the defect was observed for the first time

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Lipolysis and fatty acid degradation can be linked with astringent, hot and rancid off-tastes (McSweeney 2011) and with the formation of certain volatile compounds discussed above. The partial replacement of NaCl with KCl caused a metallic off-taste in cheeses that ripened for as long as 120 days.

CONCLUSION

Four batches with various NaCl content (from 0.64% to 1.19%) were produced, and a quarter molar NaCl replacement with KCl was included. In our work, the influence of salts on the activity of lactic acid bacteria and the formation of volatile substances, lactose fermentation, citrate metabolism, proteolysis, and lipolysis, was not observed. Contrarily, the content of salts or brining conditions affected water activity, consistency, and the contamination of cheese surface with yeasts and halotolerant microorganisms. While the typical consistency was formed only in cheeses with 1.19% NaCl acceptable salty taste was stated in cheeses with the content of salts 0.90% or higher. The partial replacement of NaCl with KCl was acceptable in cheeses ripened for shorter periods, but it caused a metallic off-taste after 120-day ripening. For the reformulation of Dutch-type cheese, the acceptance of consistency seems to be the limiting factor.

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