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BIOCHEMICAL AND MICROBIOLOGICAL CHANGES IN CHEESE INOCULATED WITH *YARROWIA LIPOLYTICA* YEAST

S u m m a r y

A *Yarrowia lipolytica* JII1c yeast strain, isolated from the Polish 'Rokpol' mould cheese, was used as an adjunct culture in the production of a Dutch-type cheese. Its effect on the microbiological and biochemical characteristics of the cheese was evaluated in this research study. Milk used to produce the cheese was inoculated with 10^5 cfu/mL yeast cells. During the ripening process, the yeast population grew systematically to reach a maximum level of 7.9 log cfu/g in the sixth week of maturation, whereas the number of lactic acid bacteria increased until the fourth week of ripening. Thereafter, the number of microorganisms in the both groups decreased. After 8 weeks of ripening, the pH value of cheese inoculated with yeasts was significantly higher than that of the control cheese sample (produced without those microorganisms) and reached the levels of 6.37 and 5.47, respectively. In the experimental cheeses, it was also found that the utilization rate of lactic and citric acids was higher. Additionally, the concentration levels of water-soluble nitrogen (WSN) and free amino groups (FAG) in the experimental cheeses were about twice as high as in the control cheese sample. A more intensive proteolysis in the experimental cheese was accompanied by a higher accumulation of biogenic amines, especially of tyramine, putrescine, and 2-phenylethylamine; in the experimental cheese, after 8 weeks, their contents amounted to: 167.01, 77.90, and 69.54 mg/100 g, respectively. In contrast, the concentration of histamine was similar in both cheeses (9.47 and 9.81 mg/100 g in the control and experimental cheese samples, respectively). Also, the experimental cheese revealed more pronounced lipolysis resulting in a higher accumulation of free fatty acids, especially of butyric, myristic, palmitic, stearic, and oleic acids. It can be concluded that the *Y. lipolytica* JII1c grew well in the cheese causing the ripening process of the cheese to significantly accelerate.

Key words: *Yarrowia lipolytica*, cheese ripening, proteolysis, lipolysis

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Introduction

Yeasts are frequently present in the form of a non-starter microflora in many types of cheese [17, 47, 48, 52, 53, 55]. The occurrence of those microorganisms in cheese is attributed to their ability to grow at a low temperature, high salt concentration levels, and a low pH level. What is more, their lipolytic and proteolytic activities and ability to assimilate or ferment lactose, and to assimilate organic acids enable them to grow in such a microenvironment [56]. The yeast population can reach a considerable quantity of cells in the cheese, up to 10^6 - 10^9 cfu/g [48]. However, their growth in cheeses varies depending on the yeast species, location in the curd, and the cheese variety [48, 56]. The prevailing yeast species found in the cheeses are: *Debaryomyces hansenii, Kluyveromyces lactis, K. fragilis, K. bulgaricus, Geotrichum candidum, Torulopsis sphaerica,* and *Saccharomyces cerevisiae* [40].

In some cheese types, the yeasts contribute to the spoilage and cause changes in the texture (gassiness, softening), flavour (fruity, bitter, or yeasty off-flavours), or colour (pigmentation or discoloration), and, in some other types, they positively affect the maturation process [15, 46, 47, 49, 53]. In particular, their positive impact on the cheese ripening process consists in the utilisation of lactic acid causing the pH level to increase and the bacterial growth to be enhanced, especially in the semi-soft cheeses with a surface film, e.g., Limburger, Tilsit, and mould ripened cheeses, such as Camembert and Roquefort [1, 14]. In the blue veined cheeses, the yeasts are assumed to enhance the development of *Penicillium roqueforti* by gas production, leading to curd openness [20, 46, 47, 48].

In addition, the growth factors produced by yeast, including pantothenic acid, riboflavin and niacin, favour the development of cheese starter microflora [4, 38]. Additionally, the proteolytic and lipolytic enzymes synthesized by the yeast may directly affect the degradation of key cheese compounds during the ripening process [19].

One of the yeast species occurring in cheese is *Yarrowia lipolytica,* but its populations are not usually numerous [26, 33, 46, 47, 55]. Our earlier investigations proved that the reason why there were low quantities of *Y. lipolytica* cells in the Polish blue-veined cheese was their vulnerability to killer toxins produced by the species that prevail in that cheese: *Candida famata* (imperfect form of *Debaryomyces hansenii*) and *C. sphaerica* [26]. On the contrary, Viljoen et al. [51], who studied South African camembert and brie cheeses, found that the *Y. lipolytica* reached an even higher population size than *D. hansenii*.

The strains of *Y. lipolytica* exhibit high proteolytic and lipolytic activities. They produce two extra-cellular proteases: alkaline serine protease and acid aspartic protease [18, 34]. The cells of *Y. lipolytica* also secrete several lipases: intracellular, membrane-bound, and extra-cellular [9]. Owing to their high hydrolytic activities, those yeasts were used as starter cultures in cheese production to accelerate the ripening process

[10, 14, 48]. However, depending on the strain used, the cheese quality significantly varied [33].

The objective of the present research study was to assess the potential of *Y. lipolytica* JII1c strain as a cheese-ripening agent. The yeast strain was selected from among other *Y. lipolytica* isolates originating from the Polish 'Rokpol' blue-veined cheese [55]. It was characterized by the highest hydrolytic activities and a resistance to toxins produced by other yeasts prevailing in the same type of cheese [28, 44, 57].

Materials and methods

Microorganism

A *Yarrowia lipolytica* JII1c yeast strain was obtained from the cultures collection of the Department of Biotechnology and Food Microbiology, Wroclaw University of Environmental and Life Sciences, where it had been previously isolated from a 'Rokpol' mould cheese [55]. The stock culture of yeast was kept on the yeast extract-malt extract agar (YM) slants at 4 °C [54].

Cheese production process

Six experimental and six control samples of Dutch-type cheeses were produced from 100 litres of milk in two successive experiments with the use of a traditional technology. Prior to producing cheese samples, the milk used was standardized to obtain 40 % of fat in dry matter, and pasteurized at 72 °C for 15 s. After cooling to 30 °C, the milk in both experimental and control cheese was inoculated with mesophilic aromatic culture (Chr. Hansen). The *Y. lipolytica* JII1c yeast co-starter was added only to the milk targeted for the experimental cheese, its amount was 5.0 log cfu/mL. The yeast inoculum was grown in a shaken culture in an YM-broth at 28 °C for 48 hours; then, the cells were counted using a haemocytometer, and collected by centrifugation (5000g, 4 ^{o}C; 15 min). Next, the cells were re-suspended in UHT milk and introduced into the cheese milk. The cheese milk was coagulated with a Maxiren preparation (Gist Brocades, Netherlands) for 30 min. Thereafter, the curds were cut into 6 - 8 mm cubes and heated to 38 °C for 15 min; then, they were pressed for 20 hours, salted in a 15 % NaCl brine for 3 hours at 10 °C, and ripened at a temperature of 15 °C and a humidity of 85 %. The cheese samples were taken for analysis immediately after salting (time 0) and, subsequently: 2, 4, 6, and 8 weeks after their ripening had started.

Microbiological analysis

The aseptically taken cheese samples (each of 10 g) were homogenized with 90 ml of a sterile solution of 2 % sodium citrate for 2 min, in a Stomacher 400 Lab Blender (Seward Medical, London, England). The successive decimal dilutions were

prepared and plated in duplicate on specific media for viable counts. The yeasts were determined on Oxytetracycline – Glucose – Yeast Extract – Agar (OGY, Oxoid). The plates were incubated at 30 °C for 5 days.

The lactic acid bacteria were counted on an M17 agar (Difco) and MRS agar (Oxoid) after the incubation at 30 °C for 3 days. The media for plating bacteria contained cycloheximide at a concentration level of 100 μg mL^{-1} to inhibit the growth of yeast.

Proximate analyses

The grated cheese samples were analysed for total solids, fat, protein, and salt concentration rates using standard methods [22, 23, 24]. The pH level of the cheese slurry was measured (1 : 1, w/v) with a pH-meter (InoLab, Germany).

Content of organic acids

The concentration rate of organic acids was measured according to Roostita & Fleet [39]. A 10 mL quantity of the water-soluble fraction (WSF) of cheese, prepared according to a method as described by Kuchroo & Fox [31], was added to 20 mL of acetonitrile, stirred for 1 min, and centrifuged. The supernatant was filtered through a 0.45 μm membrane (Millipore). The samples of 10 μL were introduced into HPLC (Agilent 1100, Agilent, USA) with an Aminex HPX-87H stainless steel column (Bio-Rad Laboratories Inc., USA). The elution of the column was performed at 55 - 60 °C using 0.07 - 0.1 % (v/v) ortophosphoric acid at a flow rate of 0.5 mL/min. The identification and quantification of the organic acids were conducted by the comparison to the pattern of standard solutions (0.5 % w/v) chromatographed under the same conditions.

Assessment of proteolysis

The content of nitrogen was determined by a micro Kjeldhal method (AOAC, 1993) using an auto 1030 Kjeltec analyzer (Kjeltec™ 2300, Foss). Water-soluble nitrogen (WSN) was determined in the water-soluble fraction (WSF) of the cheese and expressed as a percent of total nitrogen (TN). The contents of free amino acid groups in water (WSF) and the phosphotungstic acid (PTA) soluble fractions were measured using a 2,4,6-trinitrobenzenesulphonic acid (TNBS) (Sigma) [32]. A phosphotungstic acid soluble fraction of cheese was prepared from WSF [27].

The protein degradation in cheese was also analyzed by an alkaline urea-polyacrylamide gel electrophoresis [5]. The electrophoresis was performed in a dual cooled vertical slab gel electrophoresis unit SE 600 (Hoefer Scientific Instruments, San Francisco, CA, USA). The gels were stained using Comassie Brilliant Blue G250 (Sigma) [7].

Determination of the biogenic amines

Biogenic amines were extracted from the cheese at the end of the ripening period [8, 13]. The extracted amines were dansylated with a dansyl chloride (50 mg/mL in acetone). The separation of dansylated amines (5 uL) was performed at 37 °C using a column MERCK LiChroCart HPLC 3 Purospher RP-C18, 5 μm, 150 mm. The solvents were as follows: buffer pH 8.0: Tris 0.1 M pH 8.0 /acetic acid 0.1 M / water (2/1/2); solvent A: buffer pH 8.0 (30 mL) / acetonitrile (550 mL) / water (420 mL); solvent B: buffer pH 8.0 (2 mL) / acetonitrile (900 mL) / water (100 mL). The dansylated amines were detected at 254 nm. The peaks were identified by comparing their retention time and the retention time of the standard mixtures of amines. The biogenic amines were quantified using calibration curves calculated from the analysis of commercial amines mixture with the increasing concentration rates known (8 μg to 1 mg/g of cheese equivalent) in a 0.02 mol L^{-1} sulphuric acid.

Analysis of free fatty acids

Free fatty acids (FFA) were extracted from the cheese using a method by Deeth et al. [12]. Acetyl chloride was used as a methylating reagent [25]. The separation was performed using a gas chromatograph (Agilent Technologies) equipped with a mass detector (GC/MS), a capillary column (Agilent DB-224 MS), its parameters being 60 m × 250 μm × 0.25 μm. The injector temperature was changed by continuously raising it from 70 °C to 240 °C at a rate of 4 °C min^{-1}. During the entire period of investigation, the flow rate of gaseous carrier (helium) was 2.0 mL/min and the split flow ratio was 1 : 100. The peaks were identified and quantified using the FFA standard values. The relative fatty acids composition was estimated as a percentage of the total peak area.

Statistical analysis

The data were statistically analysed using a Statistica AGXP V.5.5 software. Mean values with standard deviations were reported.

Results and discussion

Microbiological characteristics

Fig. 1. shows changes in the quantities of yeast and lactic acid bacteria cells in cheeses during ripening. The yeast population in the experimental cheeses inoculated with *Y. lipolytica* JII1c systematically grew and reached the maximum level of 7.9 log cfu/g in the 6^{th} week of ripening. During the next two weeks, their quantity slightly decreased. The similar maximum yeast counts were found in the cheeses produced with four *Y. lipolytica* strains, even though they used a higher initial yeast cell concen-

tration rate of ca. 1 log cycle [33]. Other authors reported significantly lower numbers of that yeast genus in the Cheddar cheese, co-inoculated with *Y. lipolytica*; moreover, they ceased to survive after four months of ripening [14]. In the Limburger cheese, *Y. lipolytica* genus was found only during the first half of the ripening period; after that period, they were not detectable [56]. In our control cheeses, the yeasts were detected after two weeks and their maximal level was 3.6 log cfu/g at the last stage of ripening. Their colony appearance differed from easy recognizable, rough and wrinkled colonies of *Y. lipolytica.* The lactic acid bacteria exhibited a similar growth pattern in both the experimental and control cheeses. However, the number of lactic bacteria detected on M17 agar plates was higher than on MRS. Those results suggest that the starter lactococci grow better in the cheese than the leuconostocs and the non-starter lactobacilli, for which the two above named media were used.

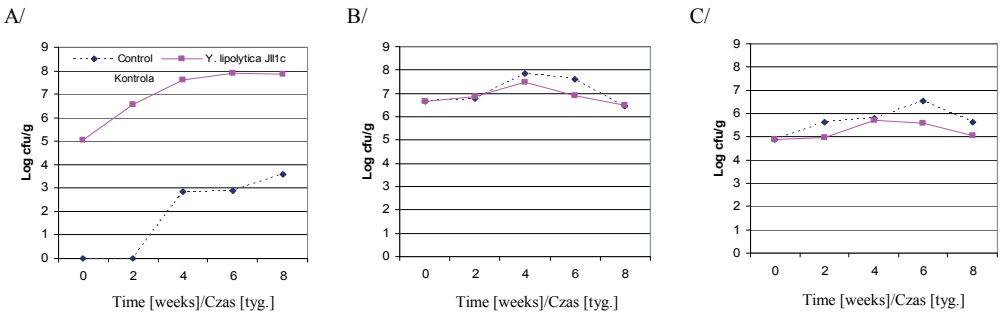

Fig. 1. Total count of micro-organisms in cheeses during ripening. (A) yeast; (B) Lactococci; (C) Lactobacilli

Rys. 1. Ogólna liczba drobnoustrojów w serach, w trakcie procesu dojrzewania. (A) drożdże, (B) paciorkowce, (C) pałeczki mlekowe.

The quantities of cells in both groups of the lactic bacteria increased until the fourth week of maturation by 1.0 - 1.5 log unit; thereafter, they declined. Generally, their populations were slightly more numerous in the control sample than in the cheeses produced with the yeast adjunct culture. Similarly, Hansen et al. [20] did not find significant differences in the counts of lactic bacteria between the Mycella cheeses maturated with or without *Saccharomyces cerevisiae* FB7 co-starter [20]. In contrast, other authors who searched into the effects of yeast adjunct cultures on the lactic acid bacteria reported a higher growth of the latter group of the microorganisms in cheeses produced with the yeasts added. The authors explained this fact by the deacidifaction effect of the cheese microenvironment caused by the yeasts and their ability to produce growth factors such as vitamins and amino acids, which stimulate the growth of lactic acid bacteria populations [4, 38].

Physicochemical characteristics

Changes in the cheese composition during ripening are summarized in Tab. 1. The total solids amount increased from 53.40 to 56.19 % and from 51.23 to 54.97 % in the control and experimental cheeses, respectively. The fat concentration in the dry matter and proteins was continuously increasing as the result of the continuously decreasing cheese moisture. Significant differences among the cheeses during ripening were found in the increases of pH values; they were about 0.9 of pH unit higher in the experimental compared to the control cheeses. After 8 weeks, the pH value in those cheeses reached a level of 6.37 and 5.47, respectively. A possible reason of a more dynamic increase in the pH value in the cheeses inoculated with yeasts could be the utilisation of the lactic acid by *Y. lipolytica*. Generally, the yeasts occurring in the cheeses contribute to the deacidification thereof by assimilating organic acids and by forming alkaline products from the lactic acid bacteria metabolites [2, 11, 37, 50,]. However, Ferreira and Viljoen [14] reported that pH in the Cheddar cheese produced with a *Y. lipolytica* yeast co-starter remained lower than in the control cheese during three months of ripening; they attributed this fact to the higher count of lactic acid bacteria to produce lactic acid at higher concentrations in that cheese [14].

T a b l e 1

Physicochemical parameters of cheeses during ripening.
Właściwości fizykochemiczne serów podczas procesu dojrzewania.

Cheese Ser	Time [weeks] Czas [tyg.]	Dry matter [DM] Sucha masa [%]	Fat Tłuszcz [%]	Fat in DM Tłuszcz w suchej masie [%]	Protein Białko [%]	NaCl [%]	Titratable acidity Kwasowość miareczkowa [°SH]	pH
Control sample Próba kontrolna	0	53.40 ± 0.09	20.51 ± 0.24	38.40 ± 0.03	28.85 ± 0.25	1.03 ± 0.02	45.6 ± 0.05	4.78 ± 0.05
	4	54.97 ± 0.27	21.93 ± 0.09	39.89 ± 0.26	29.34 ± 0.04	1.19 ± 0.25	58.7 ± 0.15	5.16 ± 0.11
	8	56.19 ± 0.36	22.72 ± 0.19	40.43 ± 0.33	30.12 ± 0.03	1.17 ± 0.03	64.8 ± 0.05	5.47 ± 0.75
Experimental cheese sample Próba badana	0	51.23 ± 0.25	20.06 ± 0.04	39.17 ± 0.01	27.33 ± 0.11	1.14 ± 0.32	44.7 ± 0.29	4.72 ± 0.30
	4	53.23 ± 0.20	21.27 ± 0.12	39.97 ± 0.16	28.74 ± 0.26	1.17 ± 0.28	78.7 ± 0.08	5.58 ± 0.31
	8	54.97 ± 0.31	22.33 ± 0.28	40.63 ± 0.31	29.64 ± 0.33	1.23 ± 0.27	113.0 ± 0.33	6.37 ± 0.18

± SD (standard deviation) / ± Odchylenie standardowe

Changes in the organic acids content in cheeses during ripening are shown in Tab. 2. Among all the acids determined (lactic, citric, propionic, acetic, succinic, and formic), the concentration rates of lactic and citric acids were the highest in the control and experimental cheeses. However, the total content of organic acids in the experimental cheeses was lower than in the control cheeses during the entire ripening process. During 8 weeks of maturation, the concentrations of those acids in the cheeses studied generally decreased. However, their decrease in the yeast-inoculated cheeses was more dynamic than in the control ones. A particularly significant decrease was reported in the contents of lactic and citric acids; this could be explained by a more intensive utilisation of these acids by the yeast co-starter; therefore, the result was a higher pH increase in these cheeses. Lactic acid generally prevails in cheeses; however, depending on the type and age of cheese, the concentrations thereof ranges between 1.94 and 17.4 mg/g cheese [6].

T a b l e 2

Contents of organic acids in cheeses during ripening.
Zawartość kwasów organicznych w serach podczas procesu dojrzewania.

Cheese Ser	Ripening time [weeks] Czas dojrzewania [tyg.]	Organic acid / Kwas organiczny [mg/100 g]					
		Lactic Mlekowy	Citric Cytrynowy	Succinic Bursztynowy	Propionic Propionowy	Acetic Octowy	Formic Mrówkowy
Control sample Próba kontrola	0	1649.7 ± 0.10	211.3 ± 0.04	3.7 ± 0.09	73.2 ± 0.25	32.0 ± 0.17	2.1 ± 0.17
	4	832.4 ± 0.17	184.6 ± 0.11	2.0 ± 0.16	62.4 ± 0.15	27.4 ± 0.25	3.4 ± 0.14
	8	154.2 ± 0.26	92.5 ± 0.18	1.8 ± 0.18	68.5 ± 0.14	21.3 ± 0.26	2.1 ± 0.04
Experimental cheese sample Próba badana	0	1357 ± 0.40	156.1 ± 0.22	3.4 ± 0.10	71.8 ± 0.35	34.5 ± 0.41	2.7 ± 0.12
	4	570 ± 0.33	92.3 ± 0.30	nd	40.5 ± 0.27	18.3 ± 0.26	1.5 ± 0.28
	8	54 ± 0.20	26.7 ± 0.13	nd	20.7 ± 0.14	13.7 ± 0.31	1.5 ± 0.15

nd – < 0.002 mg/100 g cheese; ± SD (standard deviation), nd – < 0.002 mg/100 g sera; ± SD (odychylenie standardowe)

In the cheeses under analysis, after 8 weeks of ripening, the content of lactic acid remained at a level of 154.2 and 54.0 mg/100 g in the control and experimental cheese

samples, respectively. Lactic acid is essential to properly manufacture cheese and for the ripening process. It can be further metabolized by a secondary starter and a non-starter micro-flora of cheeses [35]. Citric acid is also easily metabolized by some lactic acid bacteria to volatile flavour compounds (diacetyl, acetic acid) [35]. This acid is not detectable in some aged cheeses, especially in those of a long maturation time, such as: Emmental, Parmigiano-Reggiano [36]. Moreover, in Halloumi, a traditional Cyprian ovine cheese kept in brine, no citric acid was detected [29]. The decrease in the content of other organic acids in the cheeses analyzed was a bit surprising. Usually, their concentrations increase along with the ripening period [3, 29]. This decrease was likely owing to high fermentative activity of a starter micro-flora abundantly occurring immediately after cheese manufacture.

Proteolysis

The protein degradation during cheese ripening resulted in the increase in WSN from about 5 % in fresh cheeses to 18.20 % and 33.21 % of total nitrogen at the end of the ripening period in the control and experimental cheeses, respectively. Aljewicz et al. [2] reported comparable values. Changes in the concentrations of free amino groups in the water-soluble and PTA-soluble fractions showed the same tendency as WSN. During the whole maturation period, their content was about twice as high in the cheeses ripened with yeast co-culture compared to the control samples. After 8 weeks, the concentration rates of free amino groups in the experimental cheeses reached 8637 and 3423 μM Gly/100g in WSF and PTA fractions, respectively (Tab. 3).

A higher release of free amino groups in cheese produced with the yeast adjunct cultures was correlated with the increased production of soluble nitrogen compounds. Such a relationship was reported by a number of authors [10, 37, 56].

The protein degradation was also electrophoretically monitored (Fig. 2). The patterns of cheese proteins at different stages of ripening showed a very intensive proteolysis in the cheeses under analysis compared to the control sample cheese. After two weeks of degradation, at first, the changes were found in α_s–casein fraction. At this stage, the intensity of the band corresponding to α_s – I peptide was the highest. After four weeks, a reduction of the intensity of β-casein band was also confirmed, and it was concomitant to the increase in the bands corresponding to γ-caseins. At the end of the ripening period, the bands corresponding to the main casein fractions disappeared almost completely. In the control cheeses, most of the α_s- and β-casein remained intact until the end of ripening.

T a b l e 3

Increase in contents of WSN and free amino groups in cheese during ripening.
Przyrost azotu rozpuszczalnego i wolnych grup aminowych w serach podczas procesu dojrzewania.

Cheese Ser	Ripening time [weeks] Czas dojrzewania [tyg.]	WSN/$N_{total.}$ [%]	Free amino groups [μM Gly/100 g cheese] Wolne grupy aminowe [μM Gly/100 g sera]	
			in water soluble fraction we frakcji rozp. w wodzie	in PTA soluble fraction we frakcji rozp. w PTA
Control sample Próba kontrola	0	4.72 ± 0.17	937 ± 0.15	0
	4	9.93 ± 0.11	2542 ± 0.05	664 ± 0.31
	8	18.20 ± 0.26	4623 ± 0.19	1533 ± 0.29
Experimental cheese sample Próba badana	0	5.06 ± 0.15	943 ± 0.25	0
	4	17.20 ± 0.06	4267 ± 0.33	994 ± 0.17
	8	33.21 ± 0.19	8637 ± 0.37	3423 ± 0.33

± SD (standard deviation) / odchylenie srandardowe

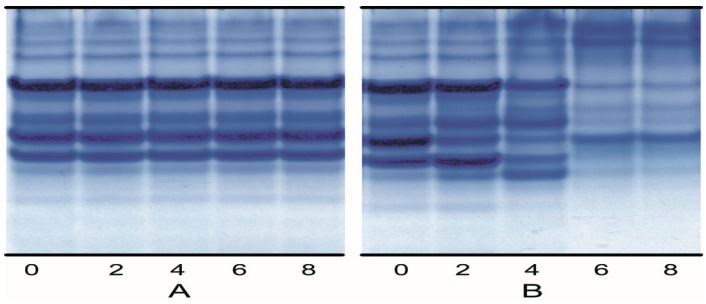

Fig. 2. Urea-PAGE of cheese proteins during ripening of (A) control cheese and (B) experimental Cheese.
Rys. 2. Rozdział elektroforetyczny białek serów podczas procesu dojrzewania. (A) ser kontrolny, (B) ser eksperymentalny.

Formation of biogenic amines

All main biogenic amines were detected in the two investigated cheeses (Tab. 4). Their concentrations increased during ripening and reached a significantly higher level in the cheeses analyzed (442.62 mg/100 g) than in the control cheeses (213.27 mg/100 g). After 8 weeks of the maturing of cheeses studied, the highest content of biogenic amines was reported in tyramine (167.01), in putrescine (77.90), and in 2-phenyl-ethylamine (69.54 mg/100 g). On the contrary, the concentration rate of histamine was

the lowest, but it was similar in both cheeses (9.47 and 9.81 mg/100 g in the control and experimental cheeses, respectively). The presence of biogenic amines in cheeses is attributed to the decarboxylating activity of microorganisms. It is confirmed that the starter cultures have a great impact on the formation of biogenic amines in cheeses; however, according to Innocente and de Agostin [21], there is no direct correlation between the microbial counts in cheese and the content of biogenic amines therein [21]. Their formation can be affected by different factors, such as: raw milk quality, composition of cheese microflora, synergism among various species, proteolysis, salt content, pH level, and ripening temperature [43, 45]. So far, the role of yeast in the production of those compounds has not been well recognized. It was found that three strains of *Y. lipolytica* originating from a Pecorino Crotonese cheese were able to decarboxylate ornithine, phenylalanine, tyrosine, and lysine, but not histidine [17, 44]. In addition, the results obtained in our previous investigations showed that the *Y. lipolytica* strains could generate the formation of biogenic amines while growing in milk [41, 44]. It was reported that the biogenic amines in the hard, Dutch-type cheese increased significantly during ripening and the tyramine was, quantitatively, the most important biogenic amine [30].

T a b l e 4

Content of biogenic amines in cheeses during ripening.
Zawartość amin biogennych w serach podczas procesu dojrzewania.

Biogenic amines [mg/100 g of cheese] Aminy biogenne [mg/100 g sera]	Control Cheese Ser kontrolny			Experimental Cheese Ser eksperymentalny		
	Ripening time [weeks] / Czas dojrzewania [tyg.]					
	0	4	8	0	4	8
TRY	nd	nd	14.84 ± 0.08	nd	7.24 ± 0.18	20.13 ± 0.21
PHE	nd	19.81 ± 0.11	36.55 ± 0.15	16.3 ± 0.05	42.15 ± 0.32	69.54 ± 0.18
PUT	nd	6.70 ± 0.09	24.01 ± 0.21	nd	25.45 ± 0.22	77.90 ± 0.13
CAD	nd	22.72 ± 0.08	27.50 ± 0.1	7.23 ± 0.16	39.21 ± 0.19	51.31 ± 0.11
HIS	nd	1.88 ± 0.27	9.47 ± 0.19	nd	nd	9.81 ± 0.09
TYR	nd	36.89 ± 0.15	59.27 ± 0.19	16.55 ± 0.3	95.30 ± 0.08	167.01 ± 0.41
SPE	nd	12.15 ± 0.11	20.11 ± 0.19	nd	11.15 ± 0.22	20.24 ± 0.14
SPN	nd	12.25 ± 0.09	21.52 ± 0.42	nd	21.13 ± 0.22	26.68 ± 0.16
Total	-	112.40	213.27	40.08	241.63	442.62

Explanatory notes: / Objaśnienia:
nd – not determined < 0.002 mg/100 g of cheese; ± SD (standard deviation); TRY – Tryptamine / Tryptamina, PHE – β-Phenylethylamine / β-Fenyloetyloamina, PUT– Putrescine / Putrescyna, CAD – Cadaweryne / Kadaweryna, HIS – Histamine / Histamina, TYR – Tyramine / Tyramina, SPE – Spermine / Spermina, SPN – Spermidyne / Spermidyna.

FFA profile

The lipolytic changes occurring during cheese maturation were monitored using the determination of the free fatty acids (FFA). Table 5 shows their individual and total contents at every stage of ripening. In most cases, the content of FFAs increased progressively during ripening. However, the degree of fatty acids released in the cheeses produced with *Y. lipolytica* JII1c was markedly higher than that found in the control cheese samples. At the end of ripening period, the total concentration rate of free fatty acids in the experimental and control cheeses were 18624 mg/kg and 9303 mg/kg, respectively. In both cheeses, it was confirmed that the concentration of oleic acid was the highest and was followed by the concentrations of palmitic, myristic, and stearic acids.

T a b l e 5

Content of free fatty acids (FFA) in cheeses during ripening.
Zawartość wolnych kwasów tłuszczowych (WKT) w serach podczas procesu dojrzewania.

FFA / WKT [mg/kg]	Control Cheese Ser kontrolny			Experimental Cheese Ser eksperymentalny		
	Ripening time [weeks] / czas dojrzewania [tyg.]					
	0	4	8	0	4	8
C_4	34 ± 0.16	192 ± 0.36	399 ± 0.26	42 ± 0.17	647 ± 0.37	1430 ± 0.41
C_6	23 ±0 .16	171 ± 0.32	238 ± 0.33	35 ± 0.27	539 ± 0.38	762 ± 0.28
C_8	32 ± 0.25	166 ± 0.27	293 ± 0.30	38 ± 0.27	183 ± 0.30	425 ± 0.33
C_{10}	63 ± 0.27	267 ± 0.25	542 ± 0.34	62 ± 0.47	246 ± 0.18	627 ± 0.27
C_{12}	53 ± 0.25	210 ± 0.24	560 ± 0.37	57 ± 0.32	538 ± 0.36	924 ± 0.41
C_{14}	134 ± 0.11	1002 ± 0.33	1473 ± 0.41	149 ± 0.31	1402 ± 0.30	2503 ± 0.26
C_{16}	426 ± 0.23	874 ± 0.36	1634 ± 0.31	489 ± 0.46	1856 ± 0.37	3806 ± 0.21
$C_{18:0}$	127 ± 0.14	621 ± 0.22	1010 ± 0.31	134 ± 0.36	1267 ± 0.33	2324 ± 0.40
$C_{18:1}$	334 ± 0.13	1304 ± 0.35	2412 ± 0.24	350 ± 0.18	2595 ± 0.37	3851 ± 0.50
$C_{18:2}$	123 ± 0.25	164 ± 0.26	734 ± 0.13	135 ± 0.16	893 ± 0.39	1964 ± 0.31
Total content of FFA	1351	4974	9297	1495	10168	18619

± SD (standard deviation / odchylenie standardowe)

A significant difference between the cheeses was in the content of short-chain fatty acids, especially the butyric and capronic acids, which were 3.6 and 3.2-times higher in the yeast-inoculated cheeses than in the control samples. This finding coincides with the results reported by other researchers who found that the inoculation with *Y. lipolytica* and its enzymes resulted in the highest increase in the contents of butyric

and caproic acids [37, 56]. Moreover, de Wit et al. [10] found the highest concentrations of oleic and palmitic acids among the long chain fatty acids and the contents of acetic and butyric acids among the volatile free fatty acids in the Cheddar cheese inoculated with *Y. lipolytica* as a single culture or with a mixed co-starter culture containing *Debaryomyces hansenii* [10]. Lanciotti et al. [33] reported that four strains of *Y. lipolytica* used in cheese production induced quantitative and qualitative differences in the content of the individual fatty acids compared to the control sample; however, the short-chain fatty acids did not differ significantly [33].

Conclusions

1. The *Y. lipolytica* JII1c yeast strain applied as an adjunct culture showed a good growth in cheeses during their ripening periods.
2. The growth of yeasts was correlated with a more intensive protein degradation, which resulted in higher contents of water-soluble nitrogen and free amino groups compared to control cheeses. However, at the same time, a higher concentration level of biogenic amines, especially of tyramine , was found in that cheese.
3. The addition of a yeast co-starter to the cheese caused the pH level to increase; this was probable owing to a more intensive utilisation of lactic and citric acids, which usually prevailed in the cheeses.
4. The yeast starter culture significantly impacted the lipolysis during cheese ripening; the consequence of this impact was a higher accumulation level of long-chain (oleic, palmitic, myristic and stearic) and short-chain free fatty acids (especially butyric and caproic) in the cheeses analysed compared to the control samples.
5. The results of the present research study have proven the *Y. lipolytica* yeasts to be a promising adjunct culture for applications in cheese production owing to their potential to accelerate the ripening process of cheeses significantly.

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ZMIANY BIOCHEMICZNE I MIKROBIOLOGICZNE W SERZE WYPRODUKOWANYM PRZY UDZIALE DROŻDŻY *YARROWIA LIPOLYTICA*

S t r e s z c z e n i e

Szczep drożdży *Yarrowia lipolytica* JII1c wyizolowany z sera Rokpol z przerostem pleśni zastosowany został jako kultura wspomagająca do produkcji sera typu holenderskiego. W pracy oceniano jego wpływ na właściwości biochemiczne wyprodukowanego sera oraz na rozwój mikroflory. Mleko użyte do produkcji sera zostało zaszczepione komórkami drożdży w ilości 10^5 jtk/mL. Podczas dojrzewania liczba drożdży systematycznie wzrastała, osiągając w szóstym tygodniu dojrzewania poziom maksymalny 7,9 log jtk/g, natomiast liczba bakterii mlekowych wzrastała jedynie do czwartego tygodnia dojrzewania. W kolejnych tygodniach dojrzewania liczba obu grup drobnoustrojów stopniowo malała. Po 8 tygodniach dojrzewania pH sera, do którego wprowadzono drożdże, było znacząco wyższe w porównaniu z serami kontrolnymi wyprodukowanymi bez udziału tych mikroorganizmów i osiągnęło wartość odpowiednio 6,37 i 5,47. W doświadczalnych serach obserwowano także intensywniejsze przemiany kwasu mlekowego i cytrynowego, a oznaczone wartości azotu rozpuszczalnego (WSN) i wolnych grup aminowych (FAG) były około 2-krotnie wyższe. Intensywniejszej proteolizie w serach otrzymanych przy udziale drożdży *Yarrowia lipolytica* towarzyszyła także wyższa zawartość amin biogennych, zwłaszcza tyraminy, putrescyny i 2-fenyloetylaminy. Ich ilości, w przeliczeniu na 100 g sera, wynosiły w 8. tygodniu dojrzewania odpowiednio 167,01, 77,90 i 69,54 mg. Stężenie histaminy w serach badanych i kontrolnych było zbliżone i wynosiło 9,81 i 9,47 mg w 100 g sera. Intensywniejszą lipolizę odnotowano w serach eksperymentalnych, co spowodowało większe stężenie wolnych kwasów tłuszczowych, zwłaszcza masłowego, mirystynowego, palmitynowego, stearynowego i oleinowego. Wykazano, że szczep drożdży *Y. lipolytica* JII1c wprowadzony do sera wpływał na przyspieszenie procesu jego dojrzewania.

Słowa kluczowe: *Yarrowia lipolytica*, dojrzewanie serów, proteoliza, lipoliza