Оцінка лактозозброджувальних дріжджів для ферментації суміші сироватки та яблучного пектину в клітковині дозволила обрати Kluyveromyces lactis 868–К як найбільш ефективні для одержання ферментованих напоїв високих ароматичних властивостей. Встановлена раціональна температура їх ферментації 30…32 °С, при якій спостерігається максимальне накопичення дріжджових клітин 70,5…71,2 млн/см^3 та етилового спирту 0,64…0,69 % об.

Ключові слова: яблучний пектин в клітковині, молочна сироватка, лактозброджуючі дріжджі, ферментований напій, ароматичні компоненти

Оценка лактоферментирующих дрожжей для смеси сыворотки и яблочного пектина в клетчатке позволила выбрать Kluyveromyces lactis 868–К как наиболее эффективные для получения ферментированного напитка с высокими ароматическими свойствами. Установлена их рациональная температура ферментации 30…32 °С, при которой наблюдается максимальное накопление дрожжевых клеток 70,5…71,2 млн/см^3 и этилового спирта 0,64…0,69 % об.

Ключевые слова: яблочный пектин в клетчатке, молочная сыворотка, ферментированный напиток, ароматические компоненты

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STUDY OF LACTOSE-FERMENTING YEASTS KLUYVEROMYCES LACTIS FOR WHEY AND APPLE PECTIN MIXTURE FERMENTATION

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1. Introduction

Nowadays simultaneous providing of saving resource technologies of high-quality dairy products with the multicomponent compositions is relevant. In addition, there is a problem of whey utilization because its volumes are growing steadily in order with the recycling for milk protein concentrates [1]. Thus, about nine liters of whey are obtained by producing each kilogram of cheese and the world whey production amounts are about 82 million metric tons [2, 3].

The prospects for increasing the utilization of secondary raw materials are closely related to the production of whey products which include not only whey but also various non-dairy functional components [4]. Despite the regulatory framework of dairy products based on whey technologies existence, the process of their implementation has its difficulties [5]. There is a number of whey processing technologies using bar membrane methods and biotransformation into carbohydrate and nitrogen-containing derivatives but the processes require special equipment [6–9]. The most economically expedient is the industrial realization of fermented whey beverage technologies which can be produced on any dairy enterprise equipment and does not require additional investment or energy [10–12].

2. Analysis of published data and problem statement

The fermented whey beverages contain valuable components of whey and secondary metabolic products obtained during fermentation such as proteins, lactic acid, ethanol, enzymes and volatile compounds [13].

Whey has a high biological value due to the amino acid content (isoleucine, leucine and valine) that stimulate specific intracellular metabolism pathways associated with protein synthesis and play a role in the hormonal response to feeding as the stimulator of insulin secretion [14]. But whey is not a balanced source of nutrients and contains high lactose content [15]. The whey fermentation is initiated by lactose hydrolysis by lactic acid bacteria (LAB). During fermentation, around 23–30 % of lactose is transformed into lactic acid. LAB do not metabolize lactose directly but by using lactose permease, it is transferred into the cells where it is hydrolyzed to glucose and galactose [16]. Some humans do not possess sufficient amounts of β-D-galactosidase in their digestive system and, therefore, are not able to digest lactose. This undigested lactose causes problems like bowel cramps and diarrhea.

In this aspect special attention is being paid to the development of fermented whey beverage technologies using the fermentation of whey and functional component mixture by lactose-fermenting yeasts. The main requirements of their production are the choice of appropriate ratio of whey and functional components, suitable starter cultures of yeasts and investigation of the fermentation according to the biochemical activity of lactose-fermenting strains.

Quality properties, the nutritional value of whey beverages can be improved by adding hydrocolloids as a functional component [17]. In our previous work, the possibility of using apple pectin in the fiber (APF) added in whey beverage technologies was shown [18]. This hydrocolloid consists

of wheat bran, apple powder and pectin, and increases the viscosity of whey. The wheat bran provides dietary fibers which modulate hunger and satiety moods, influence the glycemic, lipid and inflammatory status of consumers, and have prebiotic activity [19]. The apple powder is a rich source of carbohydrates, minerals, phenolic acids and flavonoids; it also shows high values of hydration properties such as water holding, water retention and swelling capacity as well as health–enhancing benefits such as cancer cell proliferation, lipid oxidation decrease and cholesterol lowering [20]. The pectin can reduce cholesterol, delay gastric emptying, and induce apoptosis of colon cancer cells [21].

The enzyme (β-D-galactosidase) which is used to hydrolyze the lactose in milk and dairy products is produced from yeasts *Kluyveromyces* spp. [22]. *Kluyveromyces lactis* included lactose-fermenting yeast strains which produce β-galactosidase that possesses the ability to assimilate lactose when lactose is used as a carbon source [23].

3. Aim and objectives of the study

The main aim of this work was to evaluate some lactose-fermenting yeast *Kluyveromyces lactis* strains and choose the cultivation conditions in order to obtain the most effective fermentation process of whey and apple pectin in the fiber (APF) mixture.

According to this aim, the following research objectives have been identified:

1. Study of exponential multiplication phase of lactose-fermenting *Kluyveromyces lactis* strains.

2. Yield of fermentation and rate of fermentation.

3. Evaluation of odor-active compounds in whey and APF fermented beverages.

4. Research materials

4. 1. Whey and apple pectin in the fiber (APF) mixture

The whey from cottage cheese was kindly supplied by Joint-Stock Company "Pyriatynsky dairy plant" (Pyriatyn, Poltava Oblast, Ukraine). APF was purchased from Private Business Health Food Factory "Nashe Nasledie" (Donetsk, Ukraine). APF consists of wheat bran (60 %), apple powder (38 %) and pectin (2 %). It had the following physical properties: 33.0±0.99 % solubility, 90.0±2.70 % moisture-retaining power in the water and 84.0±2.52 % moisture-retaining power in the whey.

It was formulation of a fermentation medium based on whey and APF mixture, with the following initial properties: 6.5±0.03 % dry matter, 4.6±0.02 % lactose, 1.3±0.01 % protein, pH 4.5.

4. 2. Yeast strains

Ten lactose-fermenting yeast *Kluyveromyces lactis* strains, coded 42 K, 95, 300, 304, 317, 318, 325, 469, 868–K and 2452 were kindly donated from the Culture Collection of Microorganisms and Plant Lines for Food and Agricultural Biotechnology of Institute of Food Biotechnology and Genomics National Academy of Science of Ukraine.

4. 3. Preparation of fermentation medium

The fermentation medium containing whey and APF mixture was prepared by the following procedures. 20 g of APF were mixed with 180 g of whey at a temperature of 35±5 °C for 10–15 min. The medium was then heated to a temperature of 74±2 °C for 15–20 seconds and cooled to a temperature of 30±2 °C and stored in a refrigerator at a temperature of 5 °C.

The obtained medium had the following initial properties: 8.5±0.04 % dry matter, 4.5±0.02 % reducing substances and pH 4.6.

4. 4. Preparation of the inoculum

For obtaining the inoculum, the yeasts during cultivation in aerobe conditions were grown on the streaked plates on whey and APF mixture liquid medium at a temperature of 30±2 C for 24 h. Cells of cultures in the concentration of 110^6 CFU/ml of the medium were transferred for inoculation of 2 l flasks with 1 l of whey and APF mixture, liquid medium. Fermentation in flasks was performed at a temperature of 30±2 C in a rotary shaker (220 rpm) for 24 h. Biomass was separated from the culture broth by filtration on the vacuum filter.

4. 5. Preparation of samples of whey and APF fermented beverages

1 g of wet biomass culture of each starter was added to 200 ml fermentation medium. All samples were then placed in a stationary condition in a heating chamber with the air temperature of 24 °C, 26 °C, 28 °C, 30 °C, 32 °C and 36 °C for 6±0.5, 12±0.5, 18±0.5, 24±0.5, 30±0.5 and 36±0.5 hours. Fermentation was stopped by rapid cooling to a temperature of 4±2 °C.

The samples of fermented whey without adding of APF were served as a control.

5. Research methods

The evaluation of lactose-fermenting *Kluyveromyces lactis* strains for whey and APF mixture fermentation was performed according to yeast cell concentration during fermentation, pH, CO_2 content, the content of dry matter and reducing substances, ethanol content in whey and APF fermented beverages and volatile compounds determination in distillate products.

5. 1. Yeast cell concentration determination

The yeast cell concentration (10^6 CFU/ml) was estimated by direct counting in Goryaev's chamber with Lugol solution embellishment. All parameters were evaluated in every 2 h of fermentation. All samples of fermented whey and APF beverages were prepared and measured three times.

5. 2. Ethanol content determination

The products had undergone distillation with obtaining their distillate products. The ethanol content of distillate products was determined at a temperature of 24 °C, 26 °C, 28 °C, 30 °C, 32 °C and 36 °C for 6±0.5, 12±0.5, 18±0.5, 24±0.5, 30±0.5 and 36±0.5 hours using the method 984.14 AOAC (AOAC, Washington, USA).

5. 3. Determination of odor–active compounds by gas chromatography-olfactometry

Gas chromatography-olfactometry (GC–O) was performed using an Agilent 7890A series gas chromatograph (Agilent, Palo Alto, CA, USA) equipped with flame ioniza-

tion detector (FID), sniffing port ODP3 (Agilent, Palo Alto, CA, USA), and a DB – 5MS capillary column (30 m length ×0.25 mm i. d. ×0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). The GC temperatures were controlled as follows: 40 °C (5 min); 4 °C/min to 120 °C; 16 °C/min to 220 °C (10 min). One μL of the sample of distillate products of whey and APF fermented beverages was injected in splitless mode and GC effluents were split to an FID and a sniffing port (1:1 ratio). The carrier gas was helium at a constant flow rate of 0.8 mL/min. The injector and detector temperatures were 230 °C and 250 °C, respectively.

The identification of volatile compounds including odor-active compounds was positively confirmed by comparing their retention times with those of authentic standard compounds. The retention indices (RI) values of volatile components were calculated with C_1–C_5 higher alcohols as external standards.

For quantitative analysis, the relative concentrations of odor-active compounds in 2 types of samples were analyzed by the CHROMPROCESSOR computer package (*Avangard TM, Ukraine; 2008*). The recovery % for each odor-active compound was obtained by the addition of the mixed solution containing 8 standard compounds (w/v, 100 μg/mL). Concentrations of odor-active compounds were calculated on the base of calibration and recovery.

5. 4. Statistical analysis

All data are expressed as mean values ± standard deviation (SD). Statistical differences between experimental groups were assessed by analysis of variance (ANOVA), using the COSTAT software package (Cohort Software, CA, USA). The main values were compared with LSD test ($P<0.05$).

6. Research results and discussion

6. 1. Study of exponential multiplication phase of lactose-fermenting Kluyveromyces lactis strains

The exponential multiplication phase of lactose-fermenting *Kluyveromyces lactis* strains shows the influence of the fermentation medium on their growth and reproduction. Above presented data of exponential multiplication phase of lactose-fermenting *Kluyveromyces lactis* strains are systemized in Table 1.

During cultivation in aerobic conditions the biomass yield was the highest by yeast cultivation in whey and apple pectin in the fiber mixture with *Kluyveromyces lactis* 868–K strain (71.3×10^6 CFU/ml). Conversely, *Kluyveromyces lactis* 300 strain showed the lowest fermentation potential. The yeast cell concentration has not been changed significantly for *Kluyveromyces lactis* 300 fermentation comparing with the inoculum and such fermentation was considered to be ineffective. Other lactose-fermenting yeast cell concentrations increased by 33–70 % after 24 h of fermentation compared with the inoculum.

During the alcoholic fermentation, the dynamics of CO_2 accumulation is positively correlated with the dynamics of biomass accumulation. *Kluyveromyces lactis* 868–K strain performed the most effective fermentation among 10 investigated lactose-fermenting strains. It accumulated the highest yeast cell concentration and CO_2 content, and the lowest reducing substance content at the end of fermentation.

Thus, CO_2 content was six times lower and reducing substances content was 3.6 times higher for *Kluyveromyces lactis* 300 strain than for *Kluyveromyces lactis* 868–K strain. pH values and dry matter contents have not been changed significantly after fermentation for 50 % of tested yeast strains (*Kluyveromyces lactis* stains coded 300, 304, 317, 318, 326). For other 50 % of tested yeast strains (*Kluyveromyces lactis* strain coded 42–K, 95, 868– K, 2452) pH values have been decreased from initial 4.60 to 3.50–3.85 and dry matter contents have been declined from 8.5 % to 6.3–7.5 %. The reducing substance content diminution was more considerable than dry matter content and made up 7.6–27.6 % from initial.

6. 2. Yield of fermentation and rate of fermentation

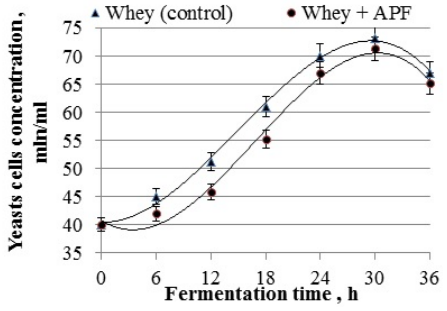
The exponential multiplication phase of *Kluyveromyces lactis* 868–K strain growth was recorded between the 6th and the 24th h of whey and APF mixture fermentation (Fig. 1, *a*). Maximum yeast cell concentration was observed on the 30 h of whey and APF mixture fermentation and control. But this parameter of whey and APF mixture fermentation was 1.59 % less than for the control (71.3×10^6 and 72.5×10^6 CFU/ml in whey and APF mixture fermentation and control, respectively). The yeast cell growth decrease for the mixture compared with the control can be associated with the presence of unfermentable dietary fiber in APF which decreases substrate accessibility.

Table 1

Physical, chemical and microbiological properties of fermented beverages based on whey and APF (n=3)

Lactose–fermenting yeast strains	Yeast cell concentration, (10^6 CFU/ml)	pH	CO_2 content, g/100 ml	Dry matter content, %	Reducing substance content, %
Kluyveromyces lactis 42 K	65.6^a(±0.09)	3.60^a(±0.00)	1.28^a(±0.00)	6.4^a(±0.01)	0.40^a(±0.00)
Kluyveromyces lactis 95	67.6^a(±0.10)	3.80^a(±0.00)	1.20^a(±0.00)	6.4^a(±0.01)	0.47^b(±0.00)
Kluyveromyces lactis 300	45.2^b(±0.17)	4.20^b(±0.01)	0.24^b(±0.00)	8.1^b(±0.03)	1.24^c(±0.02)
Kluyveromyces lactis 304	55.8^c(±1.11)	4.40^b(±0.01)	0.30^c(±0.00)	8.1^b(±0.03)	0.69^d(±0.00)
Kluyveromyces lactis 317	60.8^d(±0.36)	4.40^b(±0.01)	0.85^d(±0.00)	8.0^b(±0.03)	0.82^e(±0.01)
Kluyveromyces lactis 318	62.3^d(±0.36)	4.40^b(±0.02)	0.96^e(±0.01)	8.0^b(±0.03)	0.64^d(±0.00)
Kluyveromyces lactis 325	58.6^d(±0.34)	4.30^b(±0.02)	0.79^d(±0.00)	8.0^b(±0.03)	0.68^d(±0.00)
Kluyveromyces lactis 469	63.6^d(±0.37)	3.85^a(±0.01)	0.86^d(±0.00)	6.5^a(±0.00)	0.74^f(±0.00)
Kluyveromyces lactis 868–K	71.3^e(±1.31)	3.70^a(±0.01)	1.45^f(±0.01)	6.3^a(±0.00)	0.34^g(±0.01)
Kluyveromyces lactis 2452	62.9^d(±0.37)	3.50^a(±0.01)	0.68^g(±0.02)	7.5^b(±0.02)	0.63^d(±0.00)
$Lsd_{0.05}$	0.2290	0.0021	0.0003	0.0029	0.0006
SE±	0.189	0.00	0.00	0.00	0.00

Note: a–h Mean ±SD values having the same superscript within a column are insignificantly different (P≤0.05)



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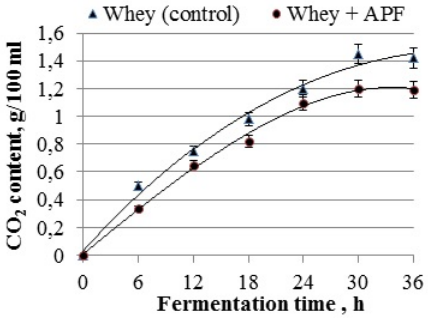


b

Fig. 1. Rate of fermentation of the medium based on whey and APF from *Kluyveromyces lactis* 868–K strain: *a* – yeast cell concentration; *b* – CO_2 content

Presented data of the rate of fermentation of the medium based on whey and APF from *Kluyveromyces lactis* 868–K strain are concordant with other investigation where *Kluyveromyces lactis* maximum biomass yield and β-galactosidase activity are achieved after 28 h of fermentation [23].

Maximum CO_2 production was observed after 30 h of fermentation (1. 2 and 1.45 g/100 ml in whey and APF mixture fermentation and control, respectively) (Fig. 1, *b*).

Yeast cell concentration didn't vary significantly at temperatures ranging from 28 to 36 °C (Fig. 2, *a*) in whey and APF mixture fermentation and control. The highest ethanol content (Fig. 2, *b*) was registered at a temperature of 32 °C in whey and APF fermented beverages and control (0.69 and 1.02 %, respectively).

According to the regulatory requirements for soft drinks (Ukraine State Standard 4069:2002) ethanol content allowed in the product is not more than 1.2 % vol. [24]. Therefore obtained results can be used to implement the technology of whey and APF fermented beverages.

6. 3. Evaluation of odor–active compounds in whey and APF fermented beverages

A total of 8 odor-active compounds were found in whey and APF fermented beverages such as acetaldehyde, methylacetate, ethylacetate, methanol, n-propane, isobutane, 2-methyl-1-butanol, 3-methyl-1-butanol. Table 2 lists their retention indices, odor descriptions, and aroma threshold values (ATVs).

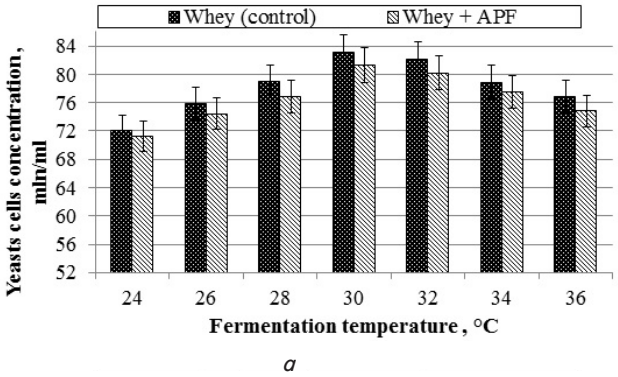
Table 2

Gas chromatography-olfactometry properties of odor-active compounds in whey and APF fermented beverages

No.	RI[a]	Components	Odor descriptions	ATV[b]
1	525	Acetaldehyde	floral, sweet	50 – 100
2	596	Methylacetate	fruit, floral	30
3	607	Ethylacetate	fruit essence	180–200
4	624	Methanol	fermented, malt	20
5	719	n-propane	vegetable, onion	100–500
6	725	Isobutane	floral, rose	100–200
7	737	2-methyl-1-butanol	fermented, malt, wine	300
8	739	3-methyl-1-butanol	fermented, malt, wine	300

Note: RI[a]; retention indices on DB–5MS column were determined using C_1–C_5 higher alcohols as external standards; ATV[b]; aroma threshold values of odor-active compounds in water (ppb) [25, 26]

The unique sensory properties of different types of fermented beverages often are due to minor differences among the volatile compounds present. By using instrumental methods for qualitative or quantitative evaluations of these differences, in addition to sensory techniques, quality assurance analysts can obtain a wealth of information about their products [28]. Differences of odor-active compounds of whey and APF fermented beverages showed the pathway of biochemical processes in whey and APF mixture fermentation and indicated the biochemical activity of lactose-fermenting yeasts Kluyveromyces lactis. Table 3 lists contents of odor-active compounds in whey and APF fermented beverages and control with ten lactose-fermenting yeasts *Kluyveromyces lactis*.



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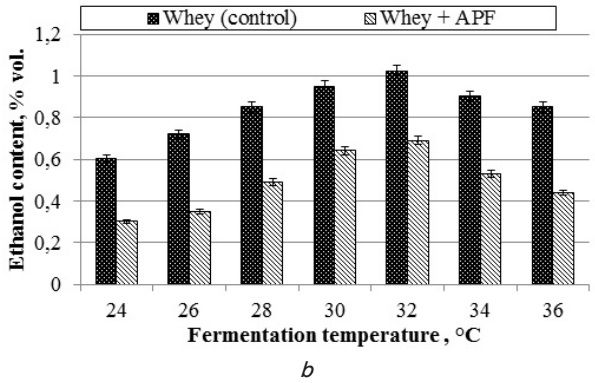

b

Fig. 2. Dependence of whey and APF mixture fermentation temperature on: *a* – yeast cell concentration; *b* – ethanol content

Table 3

Contents of odor-active compounds in whey and APF fermented beverages and control (n=3)

Lactose–fermenting yeasts	Concentrations of odor-active compounds in whey and APF fermented beverages / control*, mg/dm^3							
	Acetaldehyde	Methylacetate	Ethylacetate	Methanol	n–propane	Isobutane	2–methyl–1–butanol	3–methyl–1–butanol
Kluyveromyces lactis 42 K	169.41^{a} (±0.25)/ 204.98^{a*} (±0.31)	7.69^{a} (±0.01)/ 9.31^{b*} (±0.03)	186.12^{b} (±0.70)/ 225.20^{f*} (±1.73)	20.35^{a} (±0.03)/ 24.62^{d*} (±0.14)	127.53^{f} (±0.98)/ 154.31^{a*} (±0.23)	271.88^{e} (±5.00)/ 328.79^{h*} (±0.46)	34.89^{a} (±0.05)/ 42.21^{b*} (±0.16)	17.92^{a} (±0.02)/ 21.68^{d*} (±0.12)
Kluyveromyces lactis 95	229.04^{a} (±0.34)/ 267.97^{b*} (±0.40)	6.69^{d} (±0.03)/ 7.82^{h*} (±0.01)	195.61^{d} (±1.15)/ 228.83^{f*} (±1.76)	19.73^{b} (±0.07)/ 23.08^{a*} (±0.03)	137.53^{b} (±0.52)/ 160.91^{a*} (±0.24)	261.80^{f} (±2.01)/ 306.31^{b*} (±1.16)	20.78^{a} (±0.03)/ 24.31^{d*} (±0.14)	16.24^{b} (±0.06)/ 19.00^{e*} (±0.34)
Kluyveromyces lactis 300	164.62^{d} (±0.97)/ 177.79^{a*} (±0.27)	8.89^{b} (±0.03)/ 9.61^{d*} (±0.06)	498.17^{f} (±3.83)/ 538.02^{a*} (±0.81)	69.48^{c} (±1.38)/ 75.03^{e*} (±1.38)	133.09^{f} (±1.02)/ 143.73^{f*} (±1.11)	307.55^{f} (±2.38)/ 332.15^{d*} (±1.96)	60.72^{a} (±0.09)/ 65.57^{b*} (±0.24)	18.54^{a} (±0.02)/ 20.02^{d*} (±0.11)
Kluyveromyces lactis 304	157.80^{c} (±3.15)/ 165.69^{d*} (±0.97)	8.12^{b} (±0.03)/ 8.53^{g*} (±0.30)	473.18^{b} (±1.83)/ 496.83^{a*} (±0.74)	74.99^{h} (±0.10)/ 78.73^{f*} (±0.61)	127.32^{c} (±2.54)/ 133.68^{b*} (±0.51)	278.44^{a} (±0.41)/ 292.36^{a*} (±0.43)	54.56^{h} (±0.07)/ 57.29^{d*} (±0.34)	18.89^{b} (±0.07)/ 19.83^{b*} (±0.08)
Kluyveromyces lactis 317	159.20^{b} (±0.60)/ 167.16^{a*} (±0.25)	8.34^{d} (±0.04)/ 8.76^{h*} (±0.01)	458.42^{d} (±2.70)/ 481.34^{b*} (±1.82)	73.08^{e} (±1.34)/ 76.73^{d*} (±0.45)	128.91^{d} (±0.76)/ 135.55^{a*} (±0.20)	302.27^{b} (±1.05)/ 317.38^{a*} (±0.47)	60.72^{d} (±0.35)/ 63.75^{h*} (±0.09)	19.12^{h} (±0.02)/ 20.08^{d*} (±0.11)
Kluyveromyces lactis 318	162.90^{c} (±3.25)/ 171.05^{f*} (±1.32)	8.25^{b} (±0.03)/ 8.67^{a*} (±0.01)	494.25^{b} (±1.87)/ 518.96^{d*} (±3.06)	72.78^{b} (±0.27)/ 76.41^{a*} (±0.11)	128.67^{b} (±0.48)/ 135.11^{f*} (±1.04)	300.29^{b} (±0.33)/ 315.31^{a*} (±0.47)	44.47^{c} (±0.88)/ 46.69^{h*} (±0.07)	21.38^{c} (±0.42)/ 22.45^{e*} (±0.41)
Kluyveromyces lactis 325	166.70^{e} (±3.06)/ 179.37^{h*} (±0.25)	8.57^{f} (±0.06)/ 9.19^{d*} (±0.05)	498.47^{a} (±0.74)/ 533.36^{b*} (±2.03)	75.18^{b} (±0.28)/ 80.44^{d*} (±0.47)	123.19^{b} (±0.46)/ 137.16^{f*} (±1.06)	299.62^{f} (±2.30)/ 320.59^{a*} (±0.48)	26.24^{h} (±0.03)/ 28.07^{a*} (±0.04)	17.96^{g} (±0.64)/ 19.21^{b*} (±0.07)
Kluyveromyces lactis 469	172.48^{f} (±1.33)/ 213.47^{f*} (±1.64)	8.03^{a} (±0.01)/ 9.39^{a*} (±0.01)	202.72^{g} (±7.23)/ 237.18^{b*} (±0.90)	74.48^{a} (±0.11)/ 87.14^{b*} (±0.33)	133.29^{h} (±0.18)/ 155.94^{d*} (±0.92)	232.27^{e} (±4.27)/ 271.75^{h*} (±0.38)	18.39^{g} (±0.65)/ 21.51^{d*} (±0.12)	17.56^{e} (±0.32)/ 20.54^{h*} (±0.03)
Kluyveromyces lactis 868–K	27.00^{h} (±0.04)/ 27.81^{a*} (±0.04)	10.61^{d} (±0.06)/ 10.92^{b*} (±0.04)	85.11^{h} (±0.11)/ 87.66^{d*} (±0.51)	14.55^{d} (±0.08)/ 14.98^{h*} (±0.03)	11.84^{h} (±0.01)/ 12.55^{f*} (±0.10)	29.30^{h} (±0.04)/ 30.18^{d*} (±0.18)	73.52^{b} (±0.27)/ 75.72^{a*} (±0.11)	211.11^{d} (±1.24)/ 217.44^{b*} (±0.83)
Kluyveromyces lactis 2452	170.53^{b} (±0.64)/ 209.75^{a*} (±0.31)	9.69^{d} (±0.05)/ 11.91^{b*} (±0.04)	284.42^{a} (±0.42)/ 349.83^{h*} (±0.49)	29.33^{g} (±1.04)/ 36.07^{f*} (±0.27)	117.53^{a} (±0.17)/ 144.56^{b*} (±0.55)	291.60^{e} (±5.36)/ 358.67^{a*} (±0.54)	64.78^{a} (±0.09)/ 79.68^{b*} (±0.30)	20.52^{c} (±0.41)/ 25.23^{d*} (±0.15)
$Lsd_{0.05}$	0.6815/ 0.2880*	0.0205/ 0.0280	1.0291/ 0.6925	0.2350/ 0.1910*	0.3975/ 0.2980*	1.1575/ 0.3265*	0.1230/ 1.4580*	0.1475/ 0.1125*
SE±	1.363/ 0.576*	0.041/ 0.056*	2.058/ 1.385*	0.470/ 0.382*	0.795/ 0.596*	2.315/ 0.653*	0.246/ 0.161*	0.295/ 0.225*

Notes: a–h Mean ±SD values having the same superscript within a column are insignificantly different (P≤0.05).
** The data concerning the concentrations of odor–active compounds for control (fermented whey beverages without APF)*

The concentrations of odor–active compounds in whey and APF fermented beverages were 3–23 % less than in the control depending on the type of lactose-fermenting yeast strain used as the starter culture. As a result, the fermentation was decreased with the addition of APF. But the influence of APF was the least in the medium based on whey and APF mixture using *Kluyveromyces lactis* 868–K strain as the starter.

According to previous studies, yeasts can play a major role in the formation of diverse esters during the fermentation process. Ethyl esters, which have pleasant odor notes such as floral, fruity, have generally been reported to occur in fermented beverages. During fermentation, the esterification of ethanol and organic acids by yeasts can occur, thus conferring fruity and floral odors to fermented beverages [28]. It has been reported that the concentrations of ethyl esters depend on fermentation parameters such as yeast strains, fermentation temperature and concentration of oxygen dissolved in the medium [29]. The contents of methylacetate and ethylacetate were optimal in whey and APF fermented beverage with *Kluyveromyces lactis* 868–K strain compared with their aroma threshold values for forming of harmonious aroma (10.61 and 85.11 mg/l, respectively). There are low contents of methylacetate in whey and APF fermented beverage with *Kluyveromyces lactis* 95 and 469 strains (6.69 mg/l and 8.06 mg/l, respectively). Their fruit and floral aroma were

very mild and barely perceptible. The contents of ethylacetate in whey and APF fermented beverage with *Kluyveromyces lactis* strains coded 42 K, 95, 300, 304, 317, 318, 325, 469 and 2452 were higher in comparison with its aroma threshold values which added sharp flavor of an artificial fruit essence to whey and APF fermented beverages. These high contents indicated that the fermentation was not completed and characterized the beginning of fermentation [30].

Higher alcohols, also known as fusel alcohols, which are formed by fermentation, are important odor-active components in fermented beverages. The amyl alcohols, 3-methyl-1-butanol and 2-methyl-1-butanol, can be formed by the fermentation process from isoleucine and leucine [31]. The increased concentrations of amyl alcohols could be related to the qualities of fermented beverages due to their characteristic fermented, malt-like odor notes. So, the best content of higher alcohols and aldehydes was in whey and APF fermented beverage with *Kluyveromyces lactis* 868–K which consists of low contents of n-propane (1.84 mg/l), isobutane (29.30 mg/l), acetaldehyde (27.00 mg/l) and high contents of 2-methyl-1-butanol (73.52 mg/l) and 3-methyl-1-butanol (211.11 mg/l). In whey and APF fermented beverage with *Kluyveromyces lactis* 95 and 469, there are accumulated both the highest contents of n-propane (137.53 mg/l and 133.29 mg/l[3], respectively), isobutane (261.80 mg/l and 232.27 mg/l, respectively), acetaldehyde (229.04 mg/l and 172.48 mg/l, respectively), and low contents of 2-methyl-1-butanol (20.78 mg/l and 18.39 mg/l, respectively) and 3-methyl-1-butanol (16.24 mg/l and 17.56 mg/l, respectively) influencing the formation of general aroma. These data described incomplete fermentation too.

7. Conclusions

Based on the study of lactose-fermenting yeasts *Kluyveromyces lactis* for whey and apple pectin mixture fermentation it was established:

1. The exponential multiplication phase of lactose-fermenting *Kluyveromyces lactis* strains has shown that maximum biomass accumulation of *Kluyveromyces lactis* 868–K strain was achieved 71.3×10^6 CFU/ml compared with other investigated strains.

2. During cultivation in aerobic conditions, maximum biomass accumulation of *Kluyveromyces lactis* 868–K strain was achieved on the 30 h of cultivation at a temperature of 30±2° C. But the addition of apple pectin in the fiber into whey caused lactose–fermenting yeast growth inhibition (in exponential multiplication phase 1.59 % less biomass accumulation compared with the sample without apple pectin in the fiber). During the alcoholic fermentation, maximum CO_2 content and ethanol content of *Kluyveromyces lactis* 868–K strain were observed after 30 h of fermentation at an optimal temperature of 32° C. The highest ethanol content (0.69 %) was achieved at a temperature of 32 °C with *Kluyveromyces lactis* 868–K strain. The alcohol content is allowed for soft drinks according to the regulatory requirements.

3. The concentrations of odor-active compounds in whey and APF fermented beverages were 3–23 % less than in the control depending on lactose-fermenting yeast strains. The best contents of higher alcohols, aldehydes and esters were obtained in whey and APF fermented beverage by using *Kluyveromyces lactis* 868–K strain which consists of low contents of n-propane (1.84 mg/l), isobutane (29.30 mg/l), acetaldehyde (27 mg/l), and high contents of 2-methyl-1-butanol (73.52 mg/l), 3-methyl-1-butanol (211.11 mg/l), methylacetate (10.61 mg/l) and ethylacetate (85.11 mg/l).

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References

1. Gonzalez Siso, M. I. The biotechnological utilization of cheese whey: A review [Text] / M. I. Gonzalez Siso // Bioresource Technology. – 1996. – Vol. 57, Issue 1. – P. 1–11. doi: 10.1016/0960-8524(96)00036-3
2. Jelen, P. Whey processing. Utilization and Products [Text] / P. Jelen. – Encyclopedia of Dairy Sciences. 2nd Ed. London: London Academic Press, 2011. – 737 p.
3. Pesta, G. Utilization of whey / Utilization of by–products and treatment of waste in the food industry [Text] / G. Pesta, R. Meyer-Pittroff, W. Russ. – New York: Springer, 2007. – 1093 p. doi: 10.1007/978-0-387-35766-9
4. Baccouche, A. A physical stability study of whey–based prickly pear beverages [Text] / A. Baccouche, M. Ennouri, I. Felfoul, H. Attia // Food Hydrocolloids. – 2013. – Vol. 33, Issue 2. – P. 234–244. doi: 10.1016/j.foodhyd.2013.03.007
5. Prazeres, A. R. Cheese whey management: A review [Text] / A. R. Prazeres, F. Carvalho, J. Rivas // Journal of Environmental Management. – 2012. – Vol. 110. – P. 48–68. doi: 10.1016/j.jenvman.2012.05.018
6. Hinkova, A. Potential of Membrane Separation Processes in Cheese Whey Fractionation and Separation [Text] / A. Hinkova, P. Zidova, V. Pour, Z. Bubnik, S. Henke, A. Salova, P. Kadlec // Procedia Engineering. – 2012. – Vol. 42. – P. 1425–1436. doi: 10.1016/j.proeng.2012.07.536
7. Pan, K. A study of demineralization of whey by nanofiltration membrane [Text] / K. Pan, Q. Song, L. Wang, B. Cao // Desalination. – 2011. – Vol. 267, Issue 2-3. – P. 217–221. doi: 10.1016/j.desal.2010.09.029
8. Kargi, F. Utilization of cheese whey powder (CWP) for ethanol fermentations: Effects of operating parameters [Text] / F. Kargi, S. Ozmihci // Enzyme and Microbial Technology. – 2006. – Vol. 38, Issue 5. – P. 711–718. doi: 10.1016/j.enzmictec.2005.11.006
9. Rektor, A. Membrane filtration of Mozzarella whey [Text] / A. Rektor, G. Vatai // Desalination. – 2004. – Vol. 162. – P. 279–286. doi: 10.1016/s0011-9164(04)00052-9
10. Jelicic, I. Whey based beverages – new generation of dairy products [Text] / I. Jelicic, R. Bozanic, L. Tratnik // Mljekarstvo. – 2008. – Vol. 58. – P. 257–274.

11. Jiménez-Flores, R. Beverages based on milk fat globule membrane (MFGM) and other novel concepts for dairy- based functional beverages [Text] / R. Jiménez-Flores, I. Higuera-Ciapara, Y. Pouliot // Functional and Speciality Beverage Technology, 2009. – P. 281–296. doi: 10.1533/9781845695569.2.281
12. Harju, M. Lactose hydrolysis and other conversions in dairy products: Technological aspects [Text] / M. Harju, H. Kallioinen, O. Tossavainen // International Dairy Journal. – 2012. – Vol. 22, Issue 2. – P. 104–109. doi: 10.1016/j.idairyj.2011.09.011
13. Dragone, G. (2009). Characterisation of volatile compounds in an alcoholic beverage produced by whey fermentation [Text] / G. Dragone, S. Mussatto, J. Oliveira, J. Teixeira // Food Chemistry. – 2009. – Vol. 112, Issue 4. – P. 929–935. doi: 10.1016/j.foodchem.2008.07.005
14. Gad, A. S. Utilization Whey in Production of Functional Healthy Beverage "Whey-mango Beverages" [Text] / A. S. Gad, W. H. Emam, G. F. Mohamed, A. F. Sayd // American Journal of Food Technology. – 2013. – Vol. 8, Issue 3. – P. 133–148. doi: 10.3923/ajft.2013.133.148
15. Pescuma, M. Functional fermented whey–based beverage using lactic acid bacteria [Text] / M. Pescuma, E. M. Hebert, F. Mozzi, G. F. Valdez // International Journal of Food Microbiology. – 2010. – Vol. 141, Issue 1-2. – P. 73–81. doi: 10.1016/j.ijfoodmicro.2010.04.011
16. Baldasso, C. Concentration and purification of whey proteins by ultrafiltration [Text] / C. Baldasso, T. Barros, I. Tessaro // Desalination. – 2011. – Vol. 278, Issue 1-3. – P. 381–386. doi: 10.1016/j.desal.2011.05.055
17. Neves, A. R. Overview on sugar metabolism and its control in Lactococcus lactis – The input from in vivo NMR [Text] / A. R. Neves, W. A. Pool, J. Kok, O. P. Kuipers, H. Santos // FEMS Microbiology Reviews. – 2005. – Vol. 29, Issue 3. – P. 531–554. doi: 10.1016/j.fmrre.2005.04.005
18. Gallardo-Escamilla, F. J. Mouthfeel and flavour of fermented whey with added hydrocolloids [Text] / F. J. Gallardo-Escamilla, A. L. Kelly, C. M. Delahunty // International Dairy Journal. – 2007. – Vol. 17, Issue 4. – P. 308–315. doi: 10.1016/j.idairyj.2006.04.009
19. Grek, O. Study of effect fibers on the communication forms of moisture in mixture with milk whey [Text] / O. Grek, O. Krasulya // Maisto chemija ir technologija. – 2013. – Vol. 47. – P. 15–21
20. Prückler, M. Wheat bran–based biorefinery 1: Composition of wheat bran and strategies of functionalization [Text] / M. Prückler, S. Siebenhandlehn, S. Apprich, S. Höltinger, C. Haas, E. Schmid, W. Kneifel // LWT – Food Science and Technology. – 2014. – Vol. 56, Issue 2. – P. 211–221. doi: 10.1016/j.lwt.2013.12.004
21. Kohajdova, Z. Effect of apple pomace powder addition on farinographic properties of wheat dough and biscuits quality [Text] / Z. Kohajdova, J. Karovicova, M. Magala, V. Kuchtova // Chemical Papers. – 2014. – Vol. 68, Issue 8. – P. 1059–1065. doi: 10.2478/s11696-014-0567-1
22. Min, B. Utilization of pectin-enriched materials from apple pomace as a fat replacer in a model food system [Text] / B. Min, I. Y. Bae, H. G. Lee, S. H. Yoo, S. Lee // Bioresource Technology. – 2010. – Vol. 101, Issue 14. – P. 5414–5418. doi: 10.1016/j.biortech.2010.02.022
23. Matijevic, B. Impact of enzymatic hydrolyzed lactose on fermentation and growth of probiotic bacteria in whey [Text] / B. Matijevic, K. Lisak, R. Bozanic, L. Tratnik // Mljekarstvo. – 2011. – Vol. 61, Issue 2. – P. 154–160.
24. Lima, A. F. Comparative biochemical characterization of soluble and chitosan immobilized β–galactosidase from Kluyveromyces-lactis NRRL Y1564 [Text] / A. F. Lima, K. F. Cavalcante, M. D. Freitas, T. H. Rodrigues, M. V. Rocha, L. R. Goncalves // Process Biochemistry. – 2013. – Vol. 48, Issue 3. – P. 443–452. doi: 10.1016/j.procbio.2013.02.002
25. Ukraine State Standard 4069:2002. Soft drinks. General specifications [Text]. – Derzhspozhyvstandard of Ukraine Publishing, Kyiv, 2002. – Available at: http://dobavkam.net
26. Park, M. K. Study of volatile organic acids in freeze–dried Cheonggukjang formed during fermentation using SPME and stable–isotope dilution assay (SIDA) [Text] / M. K. Park, H.-K. Choi, D.-Y. Kwon, Y.-S. Kim // Food Chemistry. – 2007. – Vol. 105, Issue 3. – P. 1276–1280. doi: 10.1016/j.foodchem.2007.03.012
27. Azhu Valappil, Z. Impact of thermal and nonthermal processing technologies on unfermented apple cider aroma volatiles [Text] / Z. Azhu Valappil, X. Fan, H. Q. Zhang, R. L. Rouseff // Journal of Agricultural and Food Chemistry. – 2009. – Vol. 57, Issue 3. – P. 924–929. doi: 10.1021/jf803142d
28. Plutowska, B. Application of gas chromatography–olfactometry (GC–O) in analysis and quality assessment of alcoholic beverages – A review [Text] / B. Plutowska, W. Wardencki // Food Chemistry. – 2008. – Vol. 107, Issue 1. – P. 449–463. doi: 10.1016/j.foodchem.2007.08.058
29. Vanderhaegen, B. Evolution of Chemical and Sensory Properties during Aging of Top–Fermented Beer [Text] / B. Vanderhaegen, H. Neven, S. Coghe, K. J. Verstrepen, H. Verachtert, G. Derdelinckx // Journal of Agricultural and Food Chemistry. – 2003. – Vol. 51, Issue 23. – P. 6782–6790. doi: 10.1021/jf034631z
30. Duarte, W. F. Raspberry (Rubus idaeus L.) wine: Yeast selection, sensory evaluation and instrumental analysis of volatile and other compounds [Text] / W. F. Duarte, D. R. Dias, J. M. Oliveira, M. Vilanova, J. A. Teixeira, J. B. A. Silva, R. F. Schwan // Food Research International. – 2010. – Vol. 43, Issue 9. – P. 2303–2314. doi: 10.1016/j.foodres.2010.08.003
31. Marinchenko, V. O. Technology of ethanol [Text] / V. O. Marinchenko, V. A. Domaretskiy, P. L. Shiyan. – Vinnitsa: Podillay–2000, 2003. – 496 p.
32. Pino, J. A. Analysis of volatile compounds of mango wine [Text] / J. A. Pino, O. Queris // Food Chemistry. – 2011. – Vol. 125, Issue 4. – P. 1141–1146. doi: 10.1016/j.foodchem.2010.09.056