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EFFECT OF FERMENTATION CONDITIONS ON RED-BEET LEAVEN QUALITY

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Key words: red beet, leaven, lactic fermentation, borscht

The objective of this study was to determine the impact of the fermentation conditions on the quality of leavens produced from red beets and their suitability to make red-beet soup (borscht), a traditional Polish dish. Leavens fermented traditionally with a slice of rye bread as well as with the addition of *Lactobacillus plantarum* starter culture against a control sample without any additives were evaluated. On the basis of microbiological analyses, sensory evaluation of colour, taste and smell and instrumental assessment of colour and dye content, all the examined leavens were described as suitable to make borscht. All the leavens were assessed as safe for health because the level of nitrites was very low and these ions were absent in samples with the addition of *L. plantarum*. The pH level in all leavens was below 4.5, which protected them against the development of the majority of pathogenic bacteria. The sample's acidity was found to increase the quickest in samples with the addition of the starter culture.

INTRODUCTION

In recent years, a growing interest has been observed, both among consumers and food producers, in regional foods and dishes. This awareness, on the one hand, emphasises national differences but, on the other, adds some variety to our diets both in local, national and even international terms. This increased awareness is usually attributed to mass migration movements of populations resulting in a unique mixture of cultures originating from different regions meeting in the cuisine melting pot where cultures from various regions brew to produce characteristic features of national cuisine. Appropriate legislation and development of technologies which would allow production of regional dishes on a larger scale can also provide a chance for economic and touristic development of rural areas from which, as a rule, both the history and tradition of such dishes originate. This type of alternative tourism offering rest and relaxation in the bosom of nature, with regional cuisine as one of its major attractions, developed a couple of decades ago in countries of the Alps region [Hunter, 1997]. However, regional food does not only mean closeness to nature but also definite quality, hence the products used to make regional dishes should have appropriate safety certificates [Ilbery & Kncafscy, 2000].

One of the typical national Polish dishes eaten mainly in Central and Eastern Europe and which originates from different areas in Russia, Ukraine and Belarus is red beet soup prepared using leavens. Initially, the soup was prepared from the plant called cow parsnips (*Heracleum sphondylium*) but, later on, this plant was replaced by red beets. According to old Polish recipes, leaven is prepared by pickling red beets with a slice of rye bread which is to accelerate fermentation. Leavens prepared in this way are then added to vegetable-meat or vegetable (Lenten Christmas-Eve borscht) stock with a small addition of dried mushrooms which give the soup a refreshing, sour-sweet taste and beautiful ruby colour. So far, no standardised production recipes have been developed which would allow carrying out controlled fermentation of the red beet leaven as is the case with other sectors of food processing industry where selected starter cultures have been used for many years now [Halasz *et al*., 1999; Ross *et al*., 2002; Liu, 2003; Hugas *et al*., 2003].

The pickled red beet juice is an example of fermented foods which is ascribed high nutritive and taste values as well as salubrious effects. According to literature data, it is possible to produce fermented red beet juice as a source of valuable probiotic bacteria [Yoon *et al*., 2005] and it was also reported that red beet juice subjected to lactic acid fermentation is characterised by stronger anti-oxidant properties than the fresh juice [Lichtenthäler & Marx, 2005].

Red beets are also believed to possess valuable dietary and prophylactic and curing properties resulting, among others, from the fact that they contain high concentrations of minerals (Ca up to 30 mg/100g, K up to 330 mg/100 g d.m.), cellulose (1 g/100 g d.m.) [Fajkowska, 1982], folians (70–95 μg/100 g f.m.) [Jägerstad *et al*., 2005]. Red beet extracts exhibit antioxidant [Kanner *et al*., 2001; Vinson *et al*., 1998], antimutagenic [Edenharder *et al.*, 2002] and anticancerogenic properties [Kapadia *et al*., 1996]. In folk medicine, red beets have long been considered to have blood-forming and anti-tumour properties and, therefore, red beet juice is still drunk by patients after chemotherapy. This vegetable is also valued for its betalain dyes which are utilised as a red natural dye (E162) in foods. Appreciating the above-mentioned advantages of red beets, it should, however, be also stated that this vegetable tends to accumulate nitrates which,

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after reduction to nitrites, can be hazardous to human health [Kolb *et al*., 1997]. Following red beet juice lactic fermentation, it is possible to reduce by *ca*. 60% the initial nitrate content [Łaniewska-Trokenheim, 2002], while in the result of its microbiological denitrification, these compounds can be removed completely [Walkowiak-Tomczak, 2002].

The aim of the study was to determine the impact of lactic fermentation conditions on the quality of red beet leaven and its suitability for the preparation of red beet soup. The evaluation criterion of the obtained leavens concerning their culinary suitability comprised: sensory assessment of taste, smell and colour, the level of nitrites and nitrates and the pH value. The colour assessment was further expanded by instrumental measurements of dye parameters as well as by the determination of the content of betalain dyes. Fermentation was conducted with the addition of a starter culture of *Lactobacillus plantarum* T106 and employing traditional methods, *i.e.* with the addition of a slice of rye bread. The control treatment included fermentation without any inoculum and without bread.

MATERIAL AND METHODS

The quality of the obtained red beet leavens was assessed on the basis of microbiological analyses, sensory assessment of colour, taste and smell, content of betalain dyes, L*a*b* colour parameters and the content of nitrites and nitrates.

Materials. Red beets cv. Chrobry, derived from "Spójnia" Horticultural Breeding and Seed Production Ltd. in Nochów, were used in the experiments. The beets were washed, peeled and cut into slices and then put into 2000-mL sterile flasks and filled with boiled, lukewarm water at a ratio of 800 mL water: 500 g raw material, which were covered with sterile gauze. A slice of rye bread or the inoculum of *Lactobacillus plantarum* T106 bacteria were added to the above-mentioned experimental fermentation mixture, whereas the control was the mixture without any additives.

The strain of the *Lactobacillus plantarum* T106 bacteria obtained from the collection of the University of Warmia and Mazury in Olsztyn, Poland,was stored at a temperature of -80°C and prior to the fermentation, was grown on the MRS broth. The resulting biomass was centrifuged twice (10 min at 8000 g) and washed with the physiological solution preparing the inoculum of 5.0×10^7 cfu/mL which was added to the fermentation mixture at a dose of 3% v/v.

Fermentation methods. The leavens were obtained by three methods: the fermentation mixture of red beets without any additives (control), the fermentation mixture with the addition of a slice of rye bread, and the fermentation mixture inoculated with *L. plantarum*. Experiments were carried out in several series with two replications for each experimental method. The fermentation was conducted for 4 or 6 days at a temperature of 20±2°C, with leaven samples collected for analyses every 24 h.

Analytical methods. Both leavens as well as juice from fresh and pickled slices of red beets obtained using a juice extractor were assessed.

The microbiological quality of the samples examined was evaluated by determining: the total number of mesophilic bacteria (nutrient agar, P-0119, BTL, Łódź, 48 h, temperature of 37°C), the number of lactic acid bacteria (MRS medium, 24–48 h, temperature of 30°C) and the number of moulds and yeasts (wort agar, 72 h, temperature of 30°C) using the Koch's plate method.

The sensory evaluation of leavens was carried out employing the method of point scoring using both the profile and verbal scale [Baryłko-Pikielna, 1975] assessing the colour, taste and smell. The sensory evaluation was carried out on leavens after the fermentation process was terminated and after centrifugation. Next, two groups of samples were prepared: of room temperature (about 20°C) (cold juice – "leaven") and cooked samples which were prepared by boiling with the addition of an aqueous extract of spices typical of borscht (marjoram, bay, all-spice) at a temperature of *ca*. 50°C (warm leaven, further on referred to as "borscht").

The concentration of betalains dyes was determined using the spectrophotometric method according to Nilsson [Nillson, 1975]. The following values were adopted for the performed calculations: 1120 as the absorbance of 1% betaine solution measured at the wavelength of 538 nm and 750 as the absorbance of 1% vulgaxanthine solution measured at the wavelength of 476 nm.

The measurement of colour in the CIE system L*a*b* was carried out with a Hitachi U3000 spectrophotometer at 1 nm slit and scanning velocity of 1 200 nm/min, at the C light source, in transmittable light at 2 mm thickness of the optic layer. Colour was determined on the basis of the following parameters: L* (brightness), a* (proportion of red colour) and b* (proportion of yellow colour).

The content of nitrites (as NO_2 ion) and nitrates (as NO_3 ion) was determined by means of the colorimetric method with the direct cadmium reduction according to the Polish Standard [1992] and ISO Standard [1984].

In order to demonstrate the absolute content and loss of dyes, nitrites and nitrates in finished product (liquid and solid part), taking into account the dilution of the raw material with water at the ratio of 5:8, the following formula was employed:

(content in liquid part × 800 + content in slices × 500)/1300.

The results were subjected to one-way ANOVA with two variables: fermentation time, and method of fermentation. The statistical analysis was conducted using Statistica 7.1.

RESULTS AND DISCUSSION

Microbiological analyses

The mean number of lactic acid bacteria on a fresh red beet root was 3.13 log cfu/g, the number of mesophilic bacteria – 2.07 log cfu/g, whereas moulds and yeasts occurred only in some samples and – depending on the batch of the raw material – their numbers accounted for 1.52 log cfu/g.

During the fermentation process, numbers of all the examined groups of microorganisms in the leavens increased. The effect of the addition of the *L. plantarum* inoculum on the number of lactic bacteria in the course of the fermentation was visible only in the first and second day of the process when their numbers in this treatment were higher than in the control leaven and in the leaven with the addition of rye bread (Fig. 1). In the case of the finished leavens, the

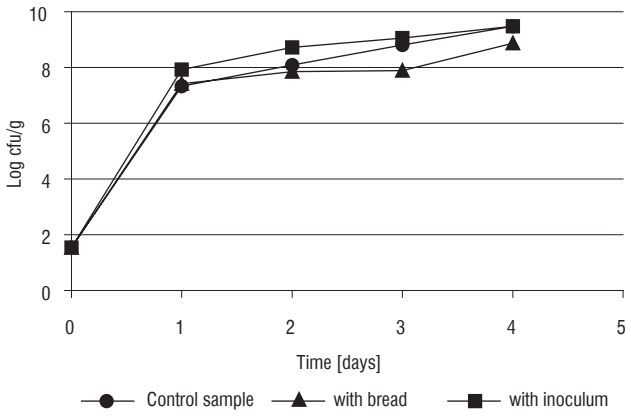

FIGURE 1. Increase of lactic acid bacteria in the course of the fermentation of red beet leaven.

numbers of lactic acid bacteria were similar in the leavens with the inoculum and the control (Table 1), ranging from 8.72–9.48 log cfu/g and 8.37–9.48 log cfu/g, respectively. In the leaven produced with the addition of rye bread, their numbers varied from 8.21 to 8.87 log cfu/g, depending on the experimental series. The influence of time and method of fermentation on the number of lactic acid bacteria was statisticaly significant ($p \leq 0.01$). In their experiments on the fermentation of red beet juice, Yoon *et al*. [2005] reported that the *L. plantarum* strain, alongside *L. acidophilus*, allowed the fastest decrease in the pH value in the juice to the safe level of 4.5 already after 48 h of culturing, whereas *L. c*asei and *L. delbrueckii* did not lower pH below 5.0 even after three days of fermentation. The pH value below 4.5 is important as it prevents the development of multiple pathogenic microorganisms, such as *Clostridium botulinum*. The bacterial counts of all the examined strains reached the level of 9 log cfu/g in the final product, *i.e.* comparable with this study.

The total number of mesophilic bacteria was similar to that of the lactic acid bacteria (Table 2). After the first 24-h period, the number of mesophilic bacteria was the highest in leavens with the addition of the inoculum, while in the ready-to-use product the concentration of this group of bacteria was the highest in the control samples or similar to samples with the addition of the starter culture. In leavens with the addition of bread, levels of both lactic acid bacteria and total number of mesophilic bacteria were in all cases the lowest. Yeasts and moulds were found sporadically, mainly in the control samples and those with the addition of bread, and their numbers ranged from 1.0 to 1.4 cfu/g.

Sensory quality

The sensory assessment was carried out on cold leavens directly after the fermentation process as well as on warm

TABLE 1. Lactic acid bacteria counts in red beet leavens after 24-h fermentation and in the finished product (after 96 or 144 h) in relation to the fermentation conditions and series of experiment.

Fermentation method	Lactic acid bacteria counts in leavens (log cfu/g)									
	Series I		Series II		Series III		Series IV		Series V	
	24 h	96 h	24 h	96 h	24 h	144 h	24 h	144 h	24 h	96 h
Control	7.33±0.18	9.48±0.13	7.41±0.50	8.87±0.07	6.71±0.21	8.92±0.06	7.55±0.39	8.37±0.17	6.81±0.07	8.80±0.12
With bread	7.41±0.11	8.87±0.11	7.15±0.33	8.50±0.06	6.66±0.12	8.74±0.11	7.16±0.69	8.23±0.33	6.58±0.24	8.21±0.37
With inoculum	7.93±0.07	9.48±0.13	7.67±0.08	8.73±0.09	7.92±0.05	8.91±0.06	8.02±0.05	9.24±0.26	7.83±0.18	8.72±0.10

Mean values ± standard deviation from 4 replications

TABLE 2. Total number of mesophillic bacteria in red beet leavens after 24-h fermentation and in the finished product (afret 96 or 144 h) in relation to the fermentation conditions and series of experiment.

Fermentation method	Total number of mesophillic bacteria in leavens (log cfu/g)									
	Series I		Series II		Series III		Series IV		Series V	
	24 h	96 h	24 h	96 h	24 h	144 h	24 h	144 h	24 h	96 h
Control	7.58±0.10	9.21±0.33	7.05±0.19	9.17±0.12	7.00±0.20	9.25±0.28	6.91±0.23	8.77±0.15	7.07±0.18	9.40±0.21
With bread	7.54±0.08	8.85±0.14	7.00±0.10	8.42±0.16	7.27±0.36	9.22±0.45	6.17±0.34	8.93±0.06	6.61±0.16	8.70±0.18
With inoculum	8.05±0.11	9.27±0.55	7.57±0.42	8.49±0.10	7.90±0.08	9.06±0.18	8.05±0.05	9.53±0.14	7.89±0.06	9.40±0.28

Mean values ± standard deviation from 4 replications

TABLE 3. Colour sensory evaluation of leavens and borschtes in relation to the fermentation method.

Colour characteristics	Control fermentation		Fermentation with bread		Fermentation with inoculum	
	Leaven	Borscht	Leaven	Borscht	Leaven	Borscht
Hue of the colour	*Red-violet*	*Violet-red*	*Pink-violet*	*Violet -pink*	*Pink-violet*	*Violet-red*
Typicality	5	5	5	5	5	5
Desirability	5	5	5	5	5	5
Intensity	5	4.25	5	4.5	5	5

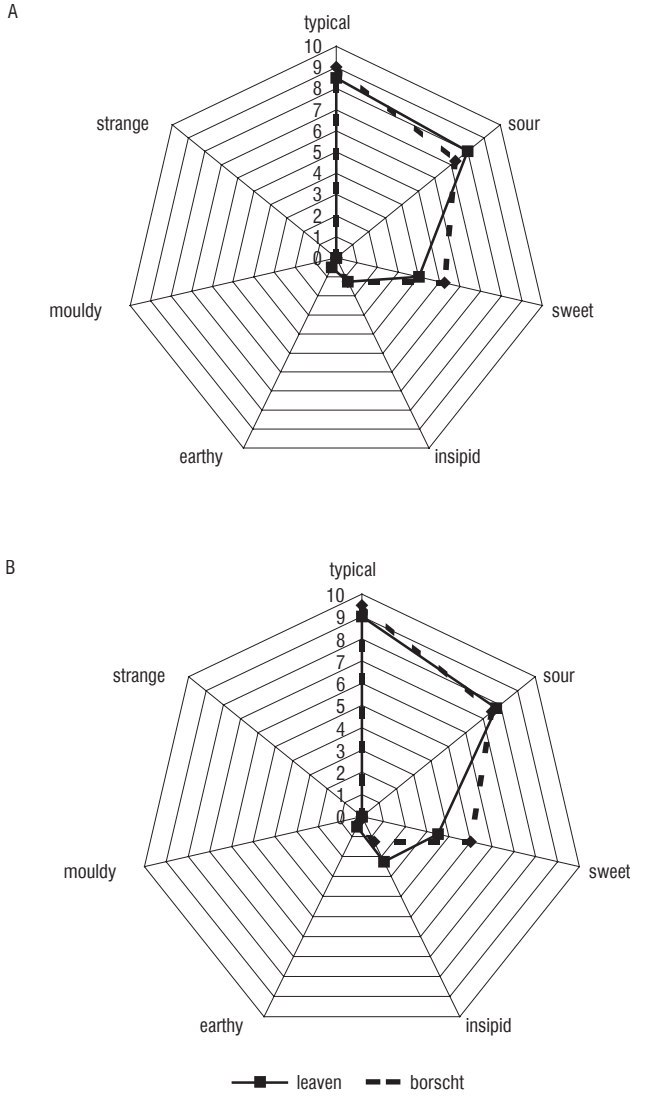

FIGURE 2. Smell and taste profilograms of red beet leaven with the addition of inoculum (A – smell, B – taste).

ones (borscht) after boiling them with the addition of spices (marjoram, bay, all-spice). In cold leavens, sweet-sour, insipid and "earthy" smell and taste typical of red beet were found dominant. In the case of warm leavens, the sense of the "earthy" and insipid taste and smell disappeared in favour of sweet-sour refreshing taste and smell (Fig. 2). The improvement observed in the taste and smell properties of warm leavens can be attributed both to the applied spice additives and evaporation of part of volatile olfactory compounds during heating. Regardless of the fermentation method, both the smell and taste of the leavens examined were evaluated as typical and desirable.

The sensory evaluation of the colour allowed concluding that in the case of cold leavens, pink-violet colour was dominant which, after their heating, changed towards pink-red or violet-red. All the examined samples received the highest scores for colour typicality and desirability, which makes them suitable for preparing borscht which should be characterised by an attractive violet-red or red colour (Table 3).

Content of betalaine dyes and colour parameters

The red beets of *Chrobry* cultivar used in these investigations were introduced into cultivation relatively recently and are characterised by a considerable content of betalaine dyes as well as a high, advantageous ratio of betacyanins to betaxanthins and high antioxidant activity in comparison with other cultivars [Mikołajczyk & Czapski, 2005].

The concentration of dyes in the leaven increased in the course of the fermentation process as a result of diffusion from the beet slices into the solution (Table 4). The ratio of red to yellow dyes increased reaching, in the ready-to-use product, the value which was higher than in the initial juice from the fresh red beet. This was connected with, among others, a drop in the pH value below 4 at which betacyanins are more stable than betaxanthins [Pátkai & Barta, 1996]. The content ratio of those dyes in the juice obtained from the pickled red beet slices was significantly lower than in the

TABLE 4. Changes in betalaine dyes and pH value in leavens during the fermentation process and in beet root juice before and after fermentation.

Sample	Part of product	Fermentation time (day)	pH	Dyes content (mg/100 g)		Red to yellow content ratio
				Red	Yellow	
Beet root juice		0	6.30	189.3±2.42	110.8±3.69	1.71±0.00
Natural leaven	Liquid part	1	6.64	0.9±0.08	1.5±0.07	0.61±0.03
		2	5.15	1.4±0.21	2.3±0.28	0.62±0.02
		3	4.13	12.6±4.00	6.3±1.32	1.95±0.28
		4	4.00	32.3±5.61	15.1±2.02	2.13±0.1
	Slices	4	4.30	49.8±0.81	49.7±1.08	1.00±0.04
Leaven with bread	Liquid part	1	6.96	0.7±0.01	0.9±0.01	0.78±0.01
		2	5.24	1.1±0.04	1.2±0.02	0.86±0.06
		3	3.98	9.6±0.11	5.9±0.49	1.63±0.16
		4	3.96	29.3±1.39	14.4±0.17	2.03±0.13
	Slices	4	4.16	46.6±1.85	43.2±1.58	1.08±0.01
Leaven with inoculum	Liquid part	1	4.59	0.7±0.10	1.5±0.07	0.52±0.10
		2	3.88	5.3±2.11	3.8±0.44	1.32±0.49
		3	3.73	10.9±1.65	4.2±0.51	2.59±0.11
		4	3.68	39.4±1.44	15.7±0.08	2.51±0.10
	Slices	4	3.86	51.1±0.41	36.9±4.62	1.40±0.20

Mean values± standard deviation; number of replications: 4

leaven and the initial juice. At the same time, it was found that in the majority of cases the value of this coefficient was the highest in leavens manufactured with the addition of the inoculum. The absolute content of dyes, both red and yellow, in the finished product was considerably lower in comparison with the raw material and these differences reached even 50% but they did not affect sensory assessment of the colour as nearly all samples were given the maximum scores for colour intensity (Tabele 3). Taking into account the dilution of the raw material with water during the fermentation process, in comparison with the control and the sample with the addition of bread, samples with the addition of the inoculum were characterised by the smallest losses (39%) of red dyes and the highest losses (44%) of yellow dyes.

The changes observed in the concentration of dyes and their mutual quantitative ratio influenced changes of colour parameters of the leavens examined (Table 5). With the increase in the content of dyes in the leaven, values of the L* parameter (*i.e.* colour lightness) decreased, whereas a* and b* values increased, which corresponds to the increase in the proportion of red and yellow dyes. It should be emphasised that the a* and b* parameters, towards the end of the fermentation, reached values higher than in the initial juice, which was due to very low values of colour constituent, especially constituent Z, in the initial juice from the fresh red beets. The X, Y and Z colour constituents serve to calculate colour parameters in the CIE Lab system but for such concentrated solutions of the dye as in the initial raw material the Lambert-Beer law is not fulfilled and the colour measurement in the transmittable light does not reflect the content of dyes but only the quality of colour.

Another factor influencing the hue tone was the pH value of about 4 (Table 4). This acidity preserved the typical, red-violet hue of the product [Pátkai & Barta, 1996], whereas the organic acids, which developed in the course of the fermentation process, gave the leavens their pleasant smell and taste. Samples to which the inoculum was added were characterised by the fastest and the strongest reduction of the leaven pH. The influence of time and method of fermentation on the pH value of the leaven was statisticaly significant ($p \leq 0.01$). However, ultimately the pH value in all leavens did not exceed 4.5, *i.e.* the microbiologically safe level. Pathogenic microorganisms are sensitive to acid environment and the pH level of 4.6 is generally accepted as the one below which *C. botulinim* – one of the most dangerous bacteria causing food poisoning – cannot develop [Czapski, 2004].

Concentrations of nitrites and nitrates

Red beets belong to a group of vegetables which tend to accumulate nitrates. According to literature data, the concentration of these ions ranges widely from several hundreds to several thousands mg/kg of fresh matter [Łaniewska-Trokenheim, 2002; Ximenes *et al*., 2000; Szymczak & Prescha, 1999]. In accordance with the Directive of the Minister of Health [2001], the acceptable concentration of nitrates in red beets is 1500 mg NO^{3-}/kg. The concentration of nitrates decreases during the process of vegetable pickling but the concentration of nitrites can increase, as a rule as the intermediate product of nitrate reduction [Kmiecik & Lisiewska, 1994]. That is why the authors of this study evaluated also the quality of the red beet leaven with regard to the content of nitrates and nitrites.

The red beets used in this study contained from 1010 to 1080 mg NO^{3-}/kg, while nitrites were not present in the raw material. In the course of the fermentation process, the concentration of nitrates in the leaven was found to increase following the transfer of soluble compounds from slices to the liquid (Table 6). Taking into account the dilution of the raw material (the solid part) by water at the ratio of 5:8, the initial nitrate concentration at the beginning of the fermenta-

TABLE 5. Changes of colour parameters in leavens during the fermentation process and in beet root juice before and after pickling.

Sample	Part of product	Fermentation time (day)	Colour parameters		
			L*	a*	b*
Beet root juice		0	11.83±0.14	45.94±0.23	43.28±0.61
Natural leaven	Liquid part	1	85.98±0.92	16.63±1.76	13.45±1.10
		2	73.75±6.37	36.39±11.23	10.65±6.65
		3	31.01±4.44	67.48±1.85	32.10±5.26
		4	21.90±0.37	59.19±1.58	38.84±3.18
	Slices	4	25.62±0.36	63.45±0.04	64.58±0.37
Leaven with bread	Liquid part	1	88.27±0.08	15.89±0.02	7.38±0.06
		2	84.98±0.74	22.58±1.09	7.32±1.27
		3	34.93±0.21	72.12±1.78	25.43±4.15
		4	26.95±0.35	66.77±0.68	37.07±0.17
	Slices	4	26.00±0.67	64.75±0.73	63.03±0.28
Leaven with inoculum	Liquid part	1	82.68±4.01	21.99±8.29	8.50±6.87
		2	48.71±5.89	76.21±5.52	0.57±4.99
		3	31.57±0.31	72.29±0.46	35.57±3.14
		4	28.27±0.33	68.81±0.31	41.84±0.80
	Slices	4	27.42±0.57	66.69±0.40	61.09±3.49

Mean values± standard deviation; number of replications: 4

TABLE 6. Changes in nitrate concentrations in the leaven and red beet slices during the fermentation process.

Sample	Part of product	Duration of fermentation (days)	Concentration of mgNO^{3-}/kg
Red beet	Juice	0	1063.2±53.72
Control fermentation	Leaven	1	72.2±31.70
		2	26.2±18.41
		3	152.0±0.31
		4	274.6±57.71
	Slices	4	418.1±57.28
Fermentation with bread	Leaven	1	121.1±27.88
		2	9.6±3.36
		3	86.9±35.12
		4	248.6±61.93
	Slices	4	360.0±67.35
Fermentation with inoculum	Leaven	1	37.5±10.97
		2	126.9±45.62
		3	211.1±62.27
		4	310.0±54.29
	Slices	4	383.9±31.31

± standard deviation from 4 replications

TABLE 7. Changes in nitrite concentrations in the leaven and red beet slices during the fermentation process.

Sample	Part of product	Duration of fermentation (days)	Concentration of mgNO^{2-}/kg
Red beet	Juice	0	0.00±0.00
Control fermentation	Leaven	1	0.00±0.00
		2	13.61±12.21
		3	2.28±2.12
		4	0.26±0.27
	Slices	4	0.00±0.00
Fermentation with bread	Leaven	1	0.01±0.01
		2	15.01±3.19
		3	0.21±0.12
		4	0.16±0.23
	Slices	4	0.00±0.00
Fermentation with inoculum	Leaven	1	0.00±0.00
		2	0.00±0.00
		3	0.00±0.00
		4	0.00±0.00
	Slices	4	0.00±0.00

± standard deviation; number from 4 replications

tion was 409 mg/kg, while after 4 days their level dropped by 17–29%. The highest NO^{3-} ion loss was recorded in the leaven samples to which bread was added. The smallest loss of the initial contents of nitrates observed in samples with the addition of the starter culture could have been caused by the fastest decrease of the pH value, because the optimal pH for the nitrate reductase as well as the remaining denitrification reductases is 7 and their activity decreases with the pH decrease [Knovles, 1982]. However, microorganisms are capable of developing a variety of mechanisms allowing them to obtain energy and a great majority of microbes utilise inorganic nitrogen (nitrate or ammonium ions), hence their depletion from the medium was associated, among others, with the assimilation processes.

In the course of the fermentation process, nitrites were identified in the brine in control samples and in samples with rye bread. Their content, on day 2 of the process, reached 13–15 mg/kg and then in the consecutive days declined (Table 7). After the fourth day of the process, the nitrite level in the substrate of the control sample amounted to 0.26 mg/kg and in the samples with the addition of bread – 0.16 mg/kg. No nitrites were present in the pickled red beet slices. In the case of the samples with the addition of the inoculum, these ions were absent both in the substrate and in slices. The solution of the problem of nitrite accumulation and the high level of nitrates found in fermented vegetable juices proposed in literature on the subject involves a combination of the process of microbiological denitrification with lactic fermentation [Emig *et al*., 1990]. The authors employed denitrification bacteria from the *Halomonas* genus and lactic acid bacteria *Leuconostoc mesenteroides* to obtain carrot juice of refreshing taste and without nitrate ions.

According to WHO/FAO [Evaluation…1980], the acceptable daily intake (ADI) for nitrates is 5 mg $NaNO_3$/kg of body weight, while the dose for nitrites – 0.2 mg $NaNO_2$/kg. This means that the acceptable dose for an average person (70 kg) amounts to about 350 mg of nitrates (255 mg of the NO_3^- ions) and 14 mg of nitrites (9 mg of the NO_2^- ions). Taking into consideration these data, it can be stated that the consumption of red beet leavens is not hazardous to health because the level of NO_2^- is low (Table 7). When average quantities of the leaven are ingested, the concentration of nitrates is not noxious either (Table 6). Some concern may be raised in the case of the nitrate content in juices obtained from fresh red beets which is frequently recommended to patients, among others, after chemotherapy in cancer treatment [Oświecimska, 1991; Madorsky, 2005]. It is evident from the research results of this study that already one glass (250 mL) of this juice covers the ADI for the NO_3^- ions. On the other hand, it is well known that in the organism of a healthy person only a small fraction of nitrates is transformed into harmful nitrites [McKnight *et al*., 1999; Amr & Hadidi, 2001]. The accumulation of nitrates by some vegetables can be partially reduced by controlling the date, dose and form of nitrogen fertilisation and applying ecological or organic farming methods [Amr & Hadidi, 2001; Rutkowska, 2005].

CONCLUSIONS

The method of preparation of red beet leavens did not affect their overall quality and culinary usefulness because all of them were assessed as suitable for the preparation of borscht. However, in the case of the leavens prepared with the addition of the *L. plantarum* culture, the pH value was found to decrease fastest reaching the lowest level in the finished product and this affected positively the final ratio of red to yellow dyes. Despite considerable losses of red (40–50%) and yellow (33–44%) dyes, all the assessed leavens were given high scores for colour intensity and desirability as a result of

the sensory evaluation performed. As a result of heating and the addition of spices, the leavens obtained (borscht) were characterised by pleasant, refreshing taste and smell with no 'earthy' aftertaste found in the initial raw material and cold leavens. The levels of nitrates (250–300 mg/kg) determined in the leavens examined do not pose any serious danger of exceeding the ADI for NO_3 at the average level of consumption. During the fermentation process, the total content of nitrates, calculated per the proportion of the liquid part and slices in the product, decreased from 17 to 30%. In the course of the fermentation process, with the exception of the leaven to which the inoculum was added, nitrites were found to occur at a level of several mg per kg. However, in the ready-to-use product, their levels ranged from 0.1 to 0.2 mg/kg, making them safe for the health of consumers.

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WPŁYW WARUNKÓW FERMENTACJI NA JAKOŚĆ ZAKWASU BURACZANEGO

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Badano jakość zakwasu z buraka ćwikłowego i jego przydatność do przygotowania barszczu, w zależności od metody fermentacji. Zakwasy fermentowane tradycyjnie z dodatkiem kromki żytniego chleba, zakwasy z dodatkiem kultury starterowej *L. plantarum* oraz zakwasy naturalne (bez dodatków) oceniano na podstawie analizy mikrobiologicznej, oceny sensorycznej barwy, smaku i zapachu, instrumentalnej oceny barwy i zawartości betalain oraz szybkości obniżania się wartości pH. Dla oceny bezpieczeństwa zdrowotnego oznaczano także zawartość azotanów (III) i (V). Sposób przygotowania zakwasu nie wpłynął na ogólną jakość i przydatność kulinarną zakwasów, wszystkie bowiem otrzymały wysokie noty w ocenie sensorycznej. Podgrzanie i dodatek przypraw pozwoliły otrzymać barszcz o przyjemnym smaku i zapachu, pozbawiony ziemistego posmaku obecnego w surowcu wyjściowym. W zakwasach z dodatkiem *L. plantarum* najszybciej następowało obniżenie pH ($p \leq 0{,}01$), co wpłynęło na zachowanie najwyższego stosunku ilościowego barwników czerwonych do żółtych (tab. 3). W próbkach tych nie stwierdzono obecności jonów azotanowych (III). Były one obecne w zakwasach z dodatkiem chleba i w próbkach kontrolnych na poziomie 0,1–0,2 mg/kg (tab. 6). W zakwasach stwierdzono obecność azotanów (V) na poziomie 250–300 mg/kg (tab. 5), co przy przeciętnym spożyciu nie stwarza zagrożenia (ADI 3,65 mg/kg masy ciała), zwłaszcza, że u zdrowego człowieka ok. 90% azotanów jest wydalanych z organizmu i nie ulega redukcji do azotanów (III).