

## Article

# Influence of Drying Type of Selected Fermented Vegetables Pomace on the Natural Colorants and Concentration of Lactic Acid Bacteria

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**Featured Application:** Powders obtained from freeze-dried fermented pomace can be used as a source of pigment and LAB. After analysis, it could be stated that future tests for drying conditions, of convective drying as well as freeze-drying, are needed to obtain higher concentrations of bacteria.



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**Abstract:** Nowadays, foods with probiotic bacteria are valuable and desired, because of their influence on human gut and health. Currently, in the era of zero waste, the food industry is interested in managing its waste. Therefore, the aim of the study was to determine the influence of drying process on the physicochemical properties of fermented vegetable pomace. The work included examining the influence of the lactic acid bacteria (*Levilactobacillus brevis*, *Lactiplantibacillus plantarum*, *Limosilactobacillus fermentum* and its mixture in the ratio 1:1:1) used for vegetable fermentation (beetroot, red pepper, carrot), obtaining pomace from fermented vegetables, and then selection of drying technique using the following methods: convection drying (CD) or freeze-drying (FD) on the physical and chemical properties of pomace. In the obtained pomace and its dried form, dry substance, water activity, color, and active substances such as betalains and carotenoids by spectrophotometric method and also bacteria concentration were evaluated. After fermentation of pomace from the same vegetable, a similar concentration of lactic acid bacteria was found as well as dry substances, color and colorants. Results of physico-chemical properties were related to the used vegetable type. After drying of pomace, it could be seen a high decrease in bacteria and colorant concentration (betalains, carotenoids) independently from drying and vegetable type as well as used starter cultures. The smallest change was observed for spontaneously fermented vegetables compared to those in which the starter culture was used.

**Keywords:** lactic acid fermentation; betalain; carotenoids; red pepper; beetroot; carrot; drying



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## 1. Introduction

The current trend to promote healthy eating is towards fermented products with probiotic properties. For this reason, fermented fruits and vegetables have become increasingly popular. The reason for this is the increase in the number of people who limit their lactose intake due to food intolerances or allergies to milk proteins. Fermented vegetable or fruit and their juices are therefore an interesting alternative for those people [1,2]. Fermentation technologies are classified as long-lasting food preservation processes, they depend mainly on: salt concentration in the brine and temperature, as well as the addition of starter strains

and the degree of multiplication of lactic acid bacteria [3]. Fermented food has a special quality, texture and taste, and has a beneficial effect on health [4,5].

In the lactic acid fermentation process, the use of starter cultures increases the chance for the desired microbiota to dominate over the other microorganisms, and to carry out a proper and controlled lactic acid fermentation process. A properly selected starter culture may also fulfill a protective and technological function, which may lead to the elimination or reduction of chemical or thermal preservation methods [6]. Traditional fermentation of vegetables depends on the microorganisms found in the raw material and is carried out spontaneously. The utilization of the LAB strain depends on the fermented matrix. Using lactic acid bacteria could introduce a potential method to improve the sensory and nutritional quality of fermented food [7]. Plant-derived LAB strains such as *Lactiplantibacillus plantarum* are most commonly used for the fermentation of the plant matrix [8–10]. LAB strains such as *Levilactobacillus brevis* or *Limosilactobacillus fermentum* can also be used for the fermentation of fruits and vegetables due to the possibility of fermentation of sugars present in these raw materials [11].

The commonly used in the fermentation process raw materials are: carrot, tomato, red bell pepper, cabbage, cucumber and beetroot. The pickled products can be eaten whole (fermented vegetables) or transferred into juices. During the production of fermented juices, one of the technologies involves fermenting whole vegetables and then pressing them for juice. During pressing, pomace is produced, which is classified as a waste from the fruit and vegetable industry. Currently, waste management in the world is heading towards transforming as much as possible into useful products, using processes involving microorganisms [12,13]. What's more, pomaces are rich in active substances such as polyphenols, natural pigments, fiber, vitamins and minerals [14–16] and LAB. One of the ways of using pomace is adding it to food, most often in a dried powdered form [17].

Drying is a basic process in the food industry [18,19]. The quality of dried products depends on the application of the appropriate drying method. With the use of suitable parameters of the drying process, such as air velocity, temperature, humidity, and by using methods that do not aerate the dried material, each of the methods can be effective [20,21]. However, dehydrated foods may still undergo adverse changes, e.g., auto-oxidation of fat, oxidation of vitamin C, discoloration, or retrogradation of starch [22]. One of the simplest methods of drying raw materials is convection/air drying, it is characterized by simplicity of construction and easy operation of devices. Convection drying (CD) is a process in which the mass and heat move simultaneously. This method is not ideal because of the nutritional value, color, appearance and taste of the dried product deteriorate during drying [20,23].

The second popular method is freeze-drying (FD). The color of freeze-dried products is more similar to the raw material from which they are made than the droughts produced with the use of other drying methods [24–26]. Freeze-drying is the most conservative way of drying vegetables and fruits as well as LAB, because the resulting product is of fairly good quality, suitable for long-term storage at ambient temperature without losing its nutritional value, as well as properties such as color, texture and aroma [27,28].

To the best of our knowledge, drying of carrot pomace [29–31], using beetroot pomace or its extracts [32–34] were investigated, however, no information about the drying of fermented vegetable pomaces are available in the literature. The aim of the study was testing the possibility to manage pomace generated during the production of juices based on fermented vegetables as a potential source of pigments and LAB. The range of the work included (1) carrying out the fermentation process of selected vegetables, using the microbiota of each vegetable or starter cultures; (2) obtaining pomace; (3) in order to obtain higher stability, drying of the pomace by convective drying or freeze-drying; (4) testing the influence of different drying type (convective and freeze-drying) on the stability of pigments and lactic acid bacteria in fermented vegetable pomaces by determination of bacteria and pigment content before and after the drying process, determination of color, dry matter and water activity in vegetable pomace.

## 2. Materials and Methods

### 2.1. Raw Materials and Microorganisms

Beetroot (*Beta vulgaris*), carrot (*Dacus carota*), and red pepper (*Capsicum annum L.*) were purchased from a local supermarket (Bronisze, Poland) and stored in a temperature range 4–6 °C maximum for 2 days before used. The strains were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and Collection of Industrial Microorganisms (KKP, Warsaw, Poland).

### 2.2. Technological Treatment

#### 2.2.1. Fermentation

##### Preparation of Inocula

Different probiotic bacterial strains (LAB), chosen after the literature survey were used: (*Levilactobacillus brevis* KKP 804, *Limosilactobacillus fermentum* KKP 811, *Lactiplantibacillus plantarum* ATCC 4080). The bacterial strains were cultured on de Man Rogosa and Sharpe Agar (MRS; Biomaxima, Poland) and incubated at 37 °C ± 1 °C for 48 h. Bacterial inocula were prepared in sterile 0.85% NaCl (*w/v*) solution to reach a population of approximately  $1 \times 10^9$  CFU/mL. The study was carried out by separately inoculating *L. brevis* (LB), *L. plantarum* (LP), and *L. fermentum* (LF) strains and a combination of tested strains in the ratio of 1:1:1 (L MIX). For spontaneous fermentation (SF) no lactic acid bacteria were added, only microbiota from vegetables was present.

##### Fermentation Process

The fermentation process was conducted in accordance with Janiszewska-Turak et al. [35] protocol. Vegetables were washed, peeled and sliced. Then were placed into glass jars to which 2% solution of NaCl in a proportion 1:1 *w/w* was added. A concentration of 1% *v/v* of the inoculum was added to the water. Jars were closed for the creation of anaerobic conditions. All experiments were left for 5–7 days depending on the vegetable (the pH in jars was tested from the 5th to 7th day until achieving a minimum pH of 3.9). After obtaining pH level, the fermentation process was stopped by placing jars into the refrigerator for at least 12 h. After a maximum of 2 days from the end, all samples were analyzed. All experiments were done in duplicate. Experiments were done in parallel.

#### 2.2.2. Juice Pressing

The fermented vegetables were used to obtain pomaces. The process was made with a juicer model NS-621CES (Kuvings, Daegu, Korea). Separately, juice and pomace were collected. Pomace was used in this research.

#### 2.2.3. Freeze-Drying

Obtained pomaces were placed on a petri dish and frozen at −40 °C (Shock Freezer HCM 51.20, Irinox, Treviso, Italy) for 5 h. Freeze drying was carried out in ALPHA 1–4 freeze dryer (Christ, Osterode, Germany) for 24 h at a heating shelf temperature of 30 °C and the constant pressure of 63 Pa, a safety pressure was set up at 103 Pa.

#### 2.2.4. Convective Drying

Convective air drying (CD: forced air circulation at the level of  $2 \text{ m s}^{-1}$ ) was performed in a laboratory convective dryer (made in our department, Warsaw, Poland) with an electronic scale (AXIS, Gdańsk, Poland). Vegetables were placed on the sieves and simultaneously heated by air with a set temperature of 45 °C.

### 2.3. Analytical Method

#### 2.3.1. Dry Matter and Water Activity

Dry matter (d.m.) was evaluated in vegetables at each stage of the process. For all samples, gravimetric method was used. About 0.6–1 g of sample was placed in a dish and dried by vacuum drying method (Memmert VO400, Schwabach, Germany) under the

pressure of 10 mPa in 75 °C for 24 h until constant weight according to information from Rybak et al. [28] and Janiszewska-Turak et al. [14]. Measurements were made in triplicate.

The water activity ( $a_w$ ) was measured at a temperature of 25 °C by a Rotronic Hygroskop DT hygrometer (Rotronic, Zurich, Switzerland). All measurements were done in triplicate.

### 2.3.2. Color Analysis

The color components were measured using a colorimeter CR-5 (Konica Minolta, Sakai Osaka, Japan) in CIE L\*a\*b\* system. The protocol of measurement and calculations was described by Rybak et al. [18]. Calibration was made with a white pattern (L\* 92.49, a\* 1.25, b\* 1.92). The measurement was done on a glass transparent petri dish on which the pomace was placed at a 5-mm layer, with standard illumination C, illuminant D65, angle of observation 2° was used. All measurements were made in five repetitions.

### 2.3.3. Betalain Analysis

Spectrophotometric method described by Janiszewska and Włodarczyk [36] and Janiszewska-Turak et al. [14] with some modifications was used to measure betalain content. For this, a spectrophotometer (Spectronic 200; Thermo Fisher Scientific Inc., Waltham, MA, USA) was used. A phosphate buffer with pH 6.5 was used for pigment extraction. Sample of approximately of 0.7 g for raw pomace or 0.05 g for dried was mixed with 50 mL of phosphate buffer for 10 min. All measurements were made in tree repetition for each sample.

The determination of betalain concentration was calculated in terms of betanin (mg betanin/100 g d.m.) and vulgaxanthin-I (mg vulgaxanthin-I/100 g d.m.). Pigment content calculations were based on the absorption values measured at 538 nm for betanin, for vulgaxanthin-I at 476 nm. The absorbance at 600 nm was measured and used to correct the amount of impurities

### 2.3.4. Carotenoids Analysis

Carotenoids were measured in accordance to methodology presented by Janiszewska-Turak and Witrowa-Rajchert [37] based on spectrophotometric measurements: 0.3 g of the grounded raw or dried sample was weighed into a centrifuge tube with the addition of distilled water (20 mL) and Carrez I and II solutions (each of 1 mL) (VWR Chemicals BDH Prolabo, Leuven, Belgium). The absorbance of the colored solutions was measured at 450 nm (Spectronic 200; Thermo Fisher Scientific Inc., Waltham, MA, USA). The total carotenoid content (TCC) was determined on the basis of the equation presented in methodology [37]. The analysis was conducted in triplicate.

### 2.3.5. Microbiological Analysis

The total count of bacteria by pour plate method was used for enumerating the viable cell LAB. In sterile conditions, 10 g of the fermented pomace samples were homogenized (Stomacher 400 Circulator, Seward, UK) for 1 min with 90 mL of 0.85% sterile sodium chloride (NaCl) [35]. Serial dilutions of the homogenates were poured into plates with MRS agar counts at 30 ± 1 °C for 48 h for lactic acid bacteria counts. The concentration of LAB was recorded as log CFU per g dry substance. The samples were analyzed in triplicate.

## 2.4. Statistical Analysis

The obtained results were subjected to a statistical analysis using the Statistica 13 software (StatSoft, Warsaw, Poland), using one-way analysis of variance with Tukey HSD test at a significance level of  $\alpha = 0.05$ . The other parameters were determined using MS Excel 16.

## 3. Results and Discussion

Physical (Table 1) and chemical properties (Tables 1 and 2), as well as microbiological analysis (Table 2) of the tested pomaces, were presented.

Table 1. General properties of beetroot, carrot and red pepper pomace.

Sample Name	Dry Matter d.m. (%)	Water Activity $a_w$ (-)	Color		
			L*	a*	b*
B	12.1 ± 0.2 b	-	13.1 ± 1.2 a	17.7 ± 2.9 efg	1.9 ± 0.8 d
B_CD	88.5 ± 1.4 c	0.42 ± 0.01 f	21.0 ± 0.7 c	11.9 ± 1.1 abc	1.9 ± 0.4 d
B_FD	97.5 ± 0.3 f	0.22 ± 0.00 b	34.0 ± 0.7 f	16.1 ± 0.3 def	5.0 ± 0.4 fgh
B_SF	5.2 ± 0.6 a	-	27.7 ± 1.7 d	23.9 ± 0.7 h	-2.8 ± 0.1 b
B_SF_CD	92.6 ± 0.8 de	0.37 ± 0.00 d	16.1 ± 2.0 ab	16.5 ± 2.1 def	0.1 ± 0.3 c
B_SF_FD	96. ± 0.13 ef	0.23 ± 0.01 b	27.7 ± 1.7 d	23.9 ± 0.7 h	-2.8 ± 0.1 b
B_LB	2.9 ± 0.6 a	-	18.3 ± 0.4 bc	14.3 ± 1.2 cde	3.9 ± 0.5 ef
B_LB_CD	90.5 ± 0.0 cd	0.41 ± 0.00 e	13. ± 1.15 a	11.6 ± 1.3 abc	2.3 ± 0.4 d
B_LB_FD	95.2 ± 1.3 ef	0.24 ± 0.01 b	31.3 ± 0.4 def	17.6 ± 0.3 def	3.3 ± 0.3 de
B_LP	9.3 ± 0.1 b	-	14.3 ± 1.8 ab	21.1 ± 2.2 gh	-1.7 ± 0.9 b
B_LP_CD	90.2 ± 2.4 cd	0.46 ± 0.01 g	16.3 ± 1.2 ab	9.2 ± 1.2 a	-2.5 ± 0.2 b
B_LP_FD	94.9 ± 0.1 ef	0.14 ± 0.00 a	29.4 ± 0.6 de	18.4 ± 0.3 fg	-5.6 ± 0.2 a
B_LF	4.0 ± 0.0 a	-	21.1 ± 0.5 c	13.1 ± 0.9 bcd	6.0 ± 0.4 h
B_LF_CD	95.3 ± 0.1 ef	0.27 ± 0.00 c	15.0 ± 7.3 ab	9.5 ± 4.7 a	3.0 ± 1.5 d
B_LF_FD	95.6 ± 0.4 ef	0.24 ± 0.00 b	32.8 ± 1.2 ef	13.3 ± 1.1 bcd	4.4 ± 0.5 efg
B_LMIX	4.6 ± 0.0 a	-	16.2 ± 0.3 ab	18.0 ± 0.7 efg	5.8 ± 0.4 gh
B_LMIX_CD	90.8 ± 1.6 cd	0.45 ± 0.01 g	14.8 ± 1.5 ab	11.5 ± 1.6 abc	1.6 ± 0.7 d
B_LMIX_FD	96.4 ± 0.2 ef	0.24 ± 0.00 b	33.6 ± 1.1 ef	17.6 ± 1.0 efg	3.2 ± 0.4 de
C	16.8 ± 0.1 a	-	45.3 ± 1.3 b	26.5 ± 0.4 bcd	34.9 ± 0.4 ghi
C_CD	89.8 ± 0.1 b	0.49 ± 0.02 c	38.1 ± 1.3 a	23.2 ± 2.0 a	23.9 ± 2.2 a
C_FD	96.7 ± 0.1 cd	0.21 ± 0.00 a	49.2 ± 0.9 c	23.3 ± 0.4 a	26.1 ± 0.6 a-d
C_SF	11.6 ± 1.1 a	-	44.2 ± 1.2 b	29.0 ± 0.2 de	32.3 ± 0.3 fgh
C_SF_CD	91.6 ± 0.1 bc	0.32 ± 0.00 b	49.5 ± 0.3 c	30.2 ± 1.2 e	29.3 ± 0.7 def
C_SF_FD	94.4 ± 0.6 bcd	0.22 ± 0.01 a	58.0 ± 1.5 d	24.4 ± 1.0 ab	25.6 ± 1.1 ab
C_LB	14.5 ± 0.6 a	-	45.2 ± 2.2 b	28.4 ± 1.8 de	34.9 ± 2.5 ghi
C_LB_CD	93.4 ± 0.4	0.35 ± 0.00 b	42.3 ± 1.9 b	30.6 ± 1.2 e	29.5 ± 1.2 def
C_LB_FD	97.9 ± 0.4 d	0.17 ± 0.01 a	58.0 ± 1.6 d	26.7 ± 0.6 b-d	30.3 ± 0.6 ef
C_LP	14.7 ± 0.0 a	-	44.3 ± 1.0 b	30.7 ± 0.3 e	36.2 ± 0.3 hi
C_LP_CD	95.0 ± 0.1 bcd	0.45 ± 0.07 c	38.7 ± 2.7 a	24.0 ± 3.7 ab	25.3 ± 5.5 ab
C_LP_FD	97.3 ± 0.7 cd	0.22 ± 0.01 a	58.1 ± 2.5 d	26.8 ± 1.7 b-e	30.5 ± 1.0 b-f
C_LF	12.9 ± 0.5 a	-	45.6 ± 1.1 b	29.2 ± 0.4 de	35.3 ± 1.0 ghi
C_LF_CD	91.6 ± 0.1 bc	0.46 ± 0.01 c	43.0 ± 1.9 b	27.8 ± 1.4 cde	27.4 ± 1.0 a-e
C_LF_FD	93.6 ± 2.7 bdc	0.14 ± 0.01 a	58.3 ± 0.5 d	24.4 ± 1.2 ab	27.6 ± 0.6 a-e
C_LMIX	11.0 ± 0.4 a	-	44.9 ± 1.2 b	29.8 ± 1.7 e	37.3 ± 1.6 i
C_LMIX_CD	89.5 ± 2.4 b	0.35 ± 0.00 b	43.9 ± 2.4 b	28.3 ± 1.9 de	29.4 ± 1.8 def
C_LMIX_FD	97.4 ± 0.1 cd	0.16 ± 0.00 a	55.6 ± 0.1 d	25.5 ± 1.0 abc	28.7 ± 0.0 b-e
P	13.0 ± 0.5 a	-	30.7 ± 1.8 abc	27.0 ± 2.7 bcd	27.9 ± 2.3 e-h
P_CD	81.9 ± 0.1 b	0.48 ± 0.07 bc	34.9 ± 1.9 cd	24.2 ± 0.6 abc	20.3 ± 1.7 abc
P_FD	91.7 ± 0.3 cd	0.19 ± 0.01 a	38.8 ± 4.8 de	20.5 ± 3.9 a	19.0 ± 2.2 a
P_SF	9.9 ± 0.5 a	-	31.0 ± 0.6 abc	31.3 ± 3.8 def	22.9 ± 2.6 a-d

Table 1. Cont.

Sample Name	Dry Matter d.m. (%)	Water Activity $a_w$ (-)	Color		
			L*	a*	b*
P_SF_CD	82.9 ± 0.4 b	0.50 ± 0.00 d	38.3 ± 1.7 de	32.2 ± 1.7 ef	25.7 ± 1.8 d–g
P_SF_FD	93.2 ± 0.0 d	0.31 ± 0.00 b	37.0 ± 3.3 de	24.5 ± 3.3 abc	26.8 ± 1.2 d–g
P_LB	8.6 ± 0.4 a	-	26.9 ± 0.8 a	30.7 ± 1.9 def	30.0 ± 1.5 ghi
P_LB_CD	83.9 ± 3.6 b	0.48 ± 0.00 c	38.3 ± 1.4 de	31.5 ± 1.5 def	23.9 ± 1.9 b–e
P_LB_FD	95.0 ± 0.1 d	0.12 ± 0.00 a	40.7 ± 4.5 ef	21.9 ± 3.5 ab	22.9 ± 3.4 a–d
P_LP	8.6 ± 0.2 a	-	28.9 ± 0.6 a	35.8 ± 1.0 f	33.1 ± 2.3 i
P_LP_CD	86.6 ± 1.7 bc	0.42 ± 0.00 c	44.6 ± 2.1 f	27.5 ± 3.1 cde	29.1 ± 2.5 e–h
P_LP_FD	96.3 ± 0.3 d	0.31 ± 0.00 b	30.4 ± 3.0 abc	26.7 ± 4.9 bcd	19.2 ± 4.8 ab
P_LF	8.4 ± 0.0 a	-	26.9 ± 1.6 a	31.5 ± 1.0 def	29.9 ± 2.3 f–i
P_LF_CD	82.5 ± 0.2 b	0.42 ± 0.00 c	37.5 ± 1.2 de	31.8 ± 0.6 def	27.8 ± 1.1 e–h
P_LF_FD	96.7 ± 0.0 d	0.31 ± 0.00 a	40.6 ± 1.8 ef	25.3 ± 1.3 abc	31.4 ± 1.8 hi
P_LMIX	9.0 ± 0.2 a	-	29.9 ± 0.8 ab	34.3 ± 1.4 f	33.4 ± 2.0 i
P_LMIX_CD	94.1 ± 0.2 d	0.44 ± 0.00 c	31.5 ± 1.1 abc	24.7 ± 0.6 abc	20.6 ± 1.2 abc
P_LMIX_FD	94.3 ± 0.0 d	0.33 ± 0.00 b	34.5 ± 5.6 bcd	23.4 ± 2.2 abc	24.6 ± 4.5 c–f

Abbreviations of sample name: B—Beet root or C—carrot or P—pepper; SF—spontaneously fermented, LB—*Levilactobacillus brevis*, LF—*Limosilactobacillus fermentum*, LP—*Lactiplantibacillus plantarum*, LMIX—*Lactobacillus* mixture of those 3 in proportion 1:1:1; CD—convective drying, FD—freeze drying; a, b . . . —different indexes for individual series mean statistically significant differences for each vegetable separately in a column at the level of  $p < 0.05$ .

Table 2. Concentration of LAB in raw or fermented pomace.

	Raw	FD	CD
	log CFU/g <sub>s.d.</sub> ± SD		
B_LB	6.70 ± 0.02	2.93 ± 0.06	<1
B_LP	6.61 ± 0.02	2.46 ± 0.12	<1
B_LF	6.63 ± 0.03	2.82 ± 0.06	<1
B_LMIX	5.06 ± 0.08	2.04 ± 0.03	<1
C_LB	6.29 ± 0.20	2.56 ± 0.06	<1
C_LP	6.05 ± 0.06	3.06 ± 0.05	<1
C_LF	6.72 ± 0.06	3.34 ± 0.04	<1
C_LMIX	5.65 ± 0.01	2.86 ± 0.03	<1
P_LB	6.65 ± 0.04	2.95 ± 0.04	<1
P_LP	7.40 ± 0.02	3.48 ± 0.04	<1
P_LF	7.16 ± 0.06	3.62 ± 0.03	<1
P_LMIX	6.07 ± 0.09	2.94 ± 0.02	<1

Abbreviations of sample name: B—beetroot or C—carrot or P—pepper; LB—*Levilactobacillus brevis*, LF—*Limosilactobacillus fermentum*, LP—*Lactiplantibacillus plantarum*, LMIX—*Lactobacillus* mixture of those 3 in proportion 1:1:1; CD—convective drying, FD—freeze drying.

### 3.1. Physical Properties of Pomace

#### 3.1.1. Pomace Water Activity ( $a_w$ ) and Dry Matter (d.m.)

From the physical properties in pomaces dry matter, water activity and color were measured. That information is needed to define the basic properties of the pomace obtained by convection drying or freeze-drying. Both the dry matter content and water activity indicate the storage properties of dried substances [38,39] while color is the main feature by which usually consumers rate the quality of the product [14,40]. Higher dry matter content and lower water activity may indicate good storage properties of the obtained product [20,41]. In pomace, dry matter ranged from 2.9–12.1 for beetroot pomace to 11.0–16.8 for raw carrot pomace. Dry matter for red pepper was placed in the middle

between those two and ranged from 8.4 to 13.0. The highest content of dry matter in all non-dried pomace was observed for pomaces obtained from raw vegetables, non-used for fermentation. The fermentation process caused a decrease in dry matter, independently from vegetable or lactobacillus strains during the fermentation process. Similar values (7.4–8.1) for dry matter of fermented pepper were observed in the research of Hallmann et al. [42] who have fermented different cultivars of pepper. It is related to the fermentation process in which the activity of LAB linked to the mineral nitrogen availability can cause intensive growth of bacteria and lead to the tissue structure loosening. That loosening structure in the final step can cause releasing from the vegetable tissue non-dissolved substances [42]. Moreover, that the loose structure of vegetables during pressing the juice can transfer more substances into the juice than leave it inside the pomace.

After the drying process, the dry matter increased in all samples. The dry matter was above the value of 89, which makes the product dry and allows the product to be included in the drought. The exception was convection-dried red pepper pomace, which, as a result of convection drying, obtained only about 82% of the dry weight. This may be related to a high sugar content in the red pepper vegetable [43], which does not allow the removal of sufficient water under the presented experimental conditions. In all experiments, dry matter obtained after freeze-drying was statistically significantly higher than those from CD, the exceptions were samples from fermented by *Limosilactobacillus fermentum* for beetroot and carrot pomace and for fermented by a mixture of *Lactobacillus* red pepper pomace for which no statistically significant difference was seen.

Moisture content (100 minus dry matter) determines how much water is in food, while water activity ( $a_w$ ) shows how that water will respond to microorganisms. The higher the water activity, the faster the growth of microorganisms (e.g., bacteria, yeasts or molds) [44,45]. It is stated that most of the microorganism activity starts when  $a_w$  is above 0.6 [46]. In the present research, a higher decrease was observed for beetroot and carrot pomaces dried in freeze dryer (Table 1). Water activity did not exceed 0.5 for the presented samples, in samples for FD water avidity was for beetroot pomaces at level 0.14–0.25, for carrot pomaces at level 0.14–0.22 and the highest for FD sample range was observed for red pepper pomaces 0.12–0.33. A water activity below 0.3 is beneficial for the stability of dried vegetables as it reduces the amount of water available for microbial growth and therefore powders can be stored longer [47]. Analyzing the samples, it could be stated that obtained after freeze-drying, dried pomace of beetroot and carrot can be treated as a stable material.

### 3.1.2. Pomace Color

Analyzing the color coefficients, it was seen that the drying process has changed the color coefficient of pomaces. For lightness, of all samples, raw or fermented with different types of LAB, a decrease after convective drying and an increase after freeze-drying were noted, an exception was raw beetroot for which after both dryings an increase was noted (Table 1). It can be related to the relatively faster degradation of the pomace top layer during convection drying, and more rapid evaporation of water from the sample surface, which could cause crust formation due to collapsing tissue walls by shrinkage [48] and partial degradation of pigments [49]. In the case of freeze-dried pomace—water is rapidly removed from the entire surface of the sample—which does not change the structure [18]. For all vegetables, beetroot, carrot and red pepper pomace drying has caused a significant decrease in coefficient  $a^*$  after CD and no changes after FD. Exception samples of red pepper pomace obtained after fermentation with *Lactiplantibacillus plantarum* and LAB mixture for them also after FD decrease was seen. For coefficient  $b^*$  of beetroot pomace, no clear correlation between drying type or used LAB in the fermentation process was noted, while for carrot and red pepper decrease in this parameter was observed. An exception was the samples obtained after spontaneous fermentation and with application of *Limosilactobacillus fermentum* for which increase in this coefficient was observed. It could be related to the low value in raw material in comparison to other raw samples. A similar observation was made for red pepper by Pinar et al. [50], who have used convective and

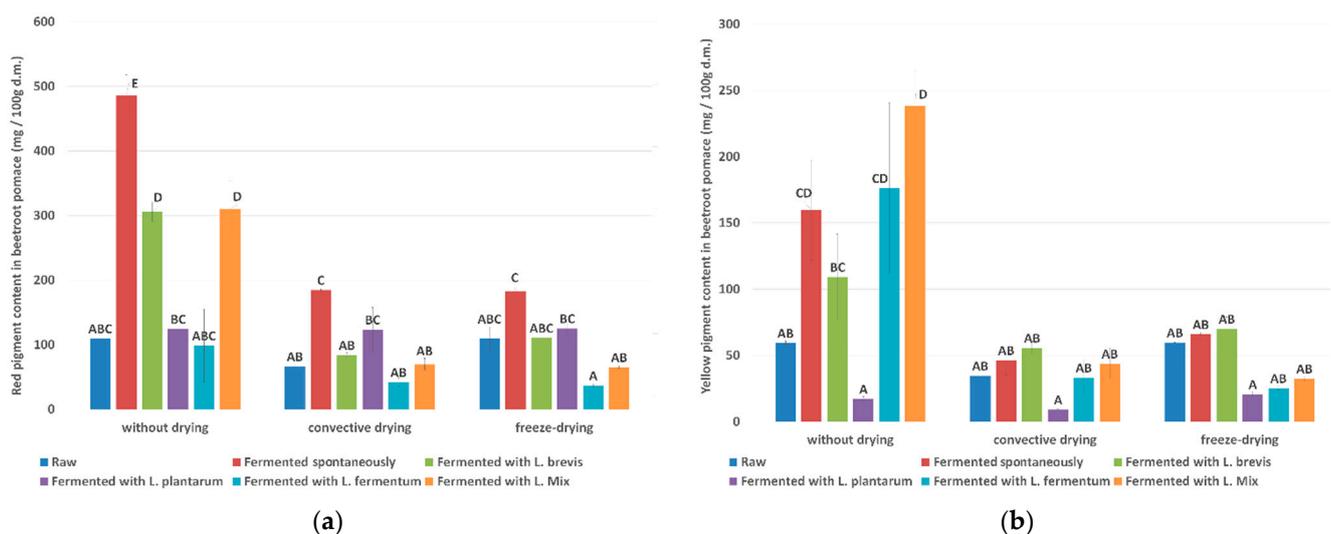
freeze-drying processes. They mentioned that the chromatic parameters were influenced by the drying type, which is related to the discoloring effect during the drying process [50].

In all analyzed samples, no relation to the fermentation process and LAB in raw samples, independently from the vegetable, was observed.

### 3.2. Pigment Content

#### 3.2.1. Pigment Content in Beetroot Pomace

Pigment content in beetroot pomaces was divided in measurement into red-violet betalain (Figure 1a) and yellow vulgaxanthin-I (Figure 1b). In the tested samples after drying process, two behaviors were observed in the pigment content. In raw beetroot pomace, pomaces fermented with *Limosilactobacillus fermentum* or *Lactiplantibacillus plantarum* no changes in red/violet pigment content were found while for the rest of the samples a significant decrease was observed. Almost the same observation was made for yellow pigment content in beetroot pomace, in raw beetroot pomace, pomaces fermented with *Lactiplantibacillus plantarum* or *Levilactobacillus brevis* where no changes were observed. For the rest of the samples, a statistically significant decrease was noted. The normal situation for the drying process is that the pigments are exposed to hot air in the CD and may also be degraded during the freeze-drying process. However, the pigments in the raw material are protected by the tissue that is not damaged during fermentation.



**Figure 1.** Pigment content in tested fermented beetroot pomace (a) Red pigment (betanin) content in beetroot pomace; (b) Yellow pigment content in fermented beetroot pomace. A, B . . . —different indexes for individual series mean statistically significant differences for given values at the level of  $p < 0.05$ .

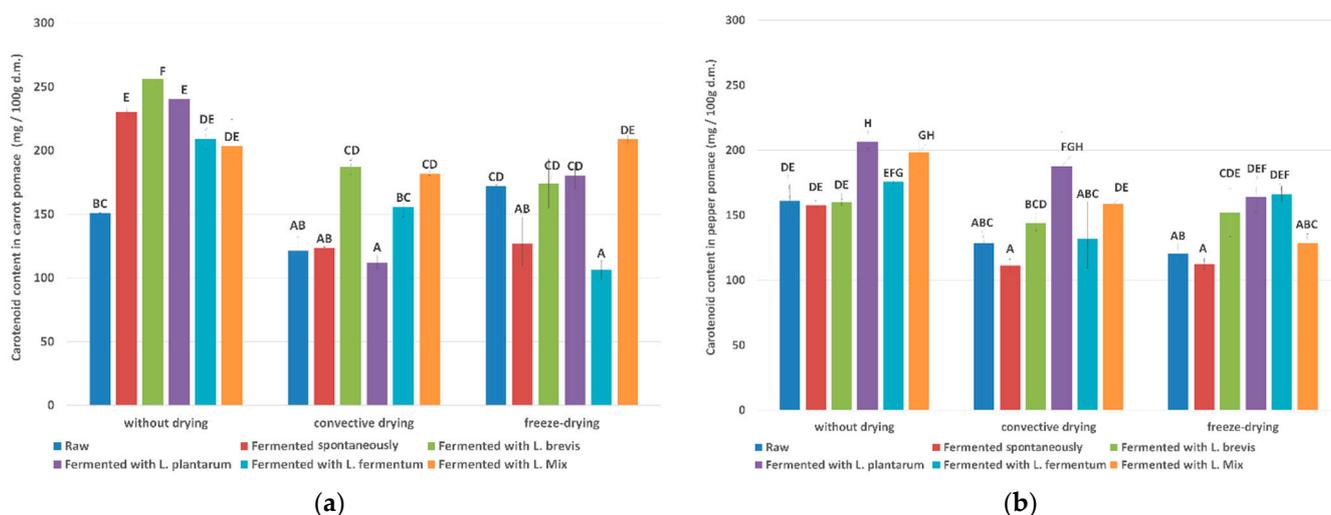
The highest degradation of red/violet pigment after drying was observed for samples with fermentation by *Lactobacillus Mix* (77% and 79% for CD and FD) and *Levilactobacillus brevis* (73% and 64% for CD and FD), while for the yellow pigment it was observed for samples *Lactobacillus Mix* (82% and 86% for CD and FD) and *Limosilactobacillus fermentum* (81% and 86% for CD and FD). The smallest degradation after drying was observed in raw beetroot samples (about 40% and 0% for CD and FD), independently from the drying type and analyzed pigment. Furthermore, a small change was observed for *Lactiplantibacillus plantarum* (1% for red/violet pigment and 48% for yellow pigment), however, the amount of red and yellow pigment was the lowest in all analyzed samples.

After the fermentation process in raw beetroot pomace, a statistically significant increase in red/violet and yellow pigment content was observed, the exception was sample fermented by *Lactiplantibacillus plantarum* in which a decrease trend in yellow and no change in red pigment was evaluated.

In fermented samples, the pH during fermentation has changed and decreased below 4. In the case of beetroot fermentation, the obtained pH below 4 favors the maintenance of betalain pigments protection for which the optimal pH is 3–6 [51–53].

### 3.2.2. Pigment Content in Carrot and Red Pepper Pomace

In carrot and in red pepper, pomace carotenoid content as an equivalent of  $\beta$ -carotene was measured. Drying of pomace of carrot and red pepper, independently from drying technique, caused a decrease in carotenoid content, the exception was a sample of carrot pomace fermented with *Lactobacillus Mix* for which no statistically significant change was found. Comparison between carrot and red pepper pomace carotenoid content showed similarity in both vegetables (Figure 2).



**Figure 2.** Pigment content in tested fermented (a) carrot pomace; (b) pepper pomace. A, B ... —different indexes for individual series mean statistically significant differences for given values at the level of  $p < 0.05$ .

The highest degradation of  $\beta$ -carotene pigment after drying was observed for carrot samples with spontaneous fermentation (46% and 45% for CD and FD), *Lactiplantibacillus plantarum* (about 53% for CD) and *L. fermentum* (about 49% for FD). In red pepper samples degradation was at level of 30–35% for raw and spontaneously fermented red pepper pomace independently from drying type and for freeze-dried pomaces with *Lactobacillus Mix*.

After the fermentation process in carrot, a statistically significant increase in pomace pigment content was observed, while for red pepper only for samples fermented with *Lactiplantibacillus plantarum* and *L. Mix* a significant increase was seen. A similar observation was mentioned by Lee et al. [54] for fermented red pepper. After fermentation process, they observed increase in the content of carotenoids (especially the volatile one) and Bartkiene et al. [55] observed the same for fermented tomato pulp by selected starter cultures, e.g., *Lactobacillus sakei* or *Pediococcus acidilactici*, in which an increase in lycopene was evaluated. Mapelli-Brahm et al. [5] have concluded that behavior of carotenoids after fermentation varies depending on the plant material and conditions of the fermentation process. Moreover, when planning the fermentation process, it should be remembered that due to enzymatic activity, bacteria can increase the extraction of carotenoids from the tissue, and what is more, the fermentation process is not related to the degradation of carotenoids [5,56].

### 3.3. Effect of Convection Drying and Freeze-Drying on the Concentration of Lactic Acid Bacteria in Pomace

Table 2 shows the effect of the drying method on the concentration of lactic acid bacteria in fermented beetroot, carrot and pepper.

In raw pomace, the concentration of LAB ranged from 5.06 to 7.40. The LAB concentration depended both on the fermented raw material (fermented pepper pomace had more LAB than beetroot and carrot) and on the bacterial species used in fermentation (in the case of fermentation using a combination of strains, the LAB concentration was lower by 1 log cycle compared to fermentation using single strains of bacteria). After drying the pomace using convective drying, the LAB concentration dropped below the detection limit. Better results were obtained after lyophilization of fermented pomace, as the decrease in the concentration of LAB was at the level of 3–4 log cycles compared to raw pomace.

Until now, fermented fruit and vegetable pomace has been investigated as a dietary component, but no attempt has been made to test the LAB concentration after the drying. It has been shown that supplementation of blueberry pomace with carbon can contribute to increasing the viability of LAB during fermentation, which in turn leads to the creation of a new type of probiotic food [57]. Moreover, fermented mulberry pomace has demonstrated the possibility of creating functional foods from LAB [58]. Unfortunately, such food cannot be stored for a long time, therefore drying the fermented pomace can extend its shelf life. However, it is necessary to select an appropriate drying method to limit the decrease in the viability of lactic acid bacteria.

#### 4. Conclusions

The food market is strongly interested to offer tasty and affordable products which would bring new functionality and health value. Therefore, a targeted fermentation and drying process of vegetable pomace offers an easy way to deliver probiotic bacteria into food products.

Our research showed that after fermentation of pomace from the same vegetable, independently from starter culture or spontaneous fermentation, a similar concentration of lactic acid bacteria was found as well as dry substances, color and colorants content. The drying process of pomace, however, caused a decrease in bacteria and colorant concentration (betalains, carotenoids) independently from drying and vegetable type as well as the used starter cultures. The slightest change was observed for spontaneously fermented vegetables in comparison to those in which the starter culture was used. Analysis of pomace physical properties indicated that freeze-dried pomaces of beetroot and carrot can be treated as a stable material with extended shelf life.

The results of the experiment showed that the fermented vegetable pomace is a promising approach towards the improvement of the functionality of food products. Obtained dried vegetable pomaces can be used in food formulations offering enrichment with pigments and probiotic bacteria with acceptable color attributes and stable quality during storage. Therefore, our findings provide a possible new direction for the design of functional products, however, further research considering the changes in sensory and polyphenolic compounds is required.

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