

## Article

# Optimization of Spontaneous Fermentation Conditions of Kohlrabi by Response Surface Methodology

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**Abstract:** Kohlrabi is a valuable crop due to its substantial amount of macro- and micronutrients. It is mostly consumed in fresh form, as jam or fermented product. This current work aimed to optimize the spontaneous fermentation conditions of kohlrabi in order to improve its product functionality and diversity. For this purpose, a Box Behnken design was employed to evaluate the effects of boiling time (0–8 min.), vinegar ratio (0–50%), and salt content (2–8%) on chemical and microbiological properties of fermented kohlrabi. Some chemical and microbiological analyses, including total phenolic content, total antioxidant capacity, total acidity, pH, salt content, total counts of yeast and molds, and mesophilic and lactic acid bacteria, were determined. The total antioxidant capacity of samples changed between 11.91 and 75.75  $\mu\text{mol Trolox}/100\text{ g}$ , respectively. Both ANOVA results ( $p < 0.05$ ) and PCA model ( $R^2 = 0.99$ ;  $Q^2 = 0.72$ ) confirmed that boiling time is the important factor affecting the fermentation process. The optimal fermentation parameters for kohlrabi were determined to be 44.12% vinegar and 2.07% salt concentrations without the boiling step by response surface methodology.

**Keywords:** kohlrabi; fermentation; response surface methodology



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## 1. Introduction

Kohlrabi (*Brassica oleracea* L.) is a vegetable that belongs to the *Brassica* family and is a good source of nutrients such as vitamin C, vitamin B<sub>6</sub>, potassium, and dietary fiber [1]. Moreover, previous studies indicated that cruciferous vegetables of all kinds (including kohlrabi) provide a healthy diet for humans due to the high levels of carotenoids, ascorbic acid, and tocopherols found in the edible parts [2,3]. Kohlrabi is rich in terms of health-promoting bioactive substances such as glucosinolates, flavonoids, phenolic acids, anthocyanins, phytosterols, and indoles, making it desirable as a functional food [4].

There have been several studies that focused on the antioxidant properties, chemical composition, and processing of kohlrabi. A study that aimed to compare the antioxidative properties of raw and thermally processed vegetables using conventional and sous vide cooking techniques suggested that the sous vide cooking method could be a good option for preserving the antioxidant properties of kohlrabi [5]. The metabolic differences between pale green and purple kohlrabi samples were investigated. It was found that purple kohlrabi contains higher levels of anthocyanins, flavonols, and phenolic acids compared to pale green kohlrabi. Moreover, both types of kohlrabi had similar levels of glucosinolates, which are bioactive compounds known for their health-promoting properties [6]. Researchers observed that high hydrostatic pressure (HHP) and thermal treatment could be used to modify the textural properties of pickled kohlrabi for specific purposes [7]. The sliced kohlrabi samples were deep fried and the color values of the slices significantly changed from light green to brown throughout the frying process [8]. The lactic acid fermentation of Chinese kohlrabi resulted in the accumulation of different volatile flavor

compounds, including aldehydes, ketones, and esters during fermentation [9]. It was suggested that UVB radiation could be used as a method to enhance the nutritional and functional properties of purple kohlrabi sprouts in previous research [10]. Kohlrabi fermentation generated notable changes in the metabolite profiles, particularly an increase in the levels of different organic acids, amino acids, and other metabolites [11].

Lactic acid fermentation is a type of fermentation used in the food industry for the production of kefir, yogurt, pickles, sauerkraut, and kimchi, as well as other fermented foods and beverages. The process involves the activity of lactic acid bacteria (LAB) converting carbohydrates, such as lactose in milk or glucose in vegetables into lactic acid. The lactic acid causes the pH of the food to drop, which helps to prevent the growth of pathogen bacteria and food spoilage. Additionally, LAB can produce a variety of flavor compounds, which contribute to the characteristic taste and aroma of fermented foods. There are many different species of LAB used in food fermentation, including, for instance, *Lactobacillus acidophilus*, *Lactocaseibacillus casei*, *L. delbrueckii*, *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, *Streptococcus thermophilus*, and *Leuconostoc mesenteroides*, etc. Moreover, probiotic LAB includes *Bifidobacterium bifidum*, *B. lactis*, *L. acidophilus*, and *S. thermophilus* species. Fermented foods are often recognized as functional foods due to their health benefits, such as improved digestion and regulation of the immune system, due to the presence of probiotics and other bioactive compounds [12–14].

Functional foods are defined as foods that are modified or designed to provide additional health benefits beyond basic nutrition. These foods consist of bioactive compounds having positive effects on human health, such as lowering disease risk or improving overall wellness. Functional foods, such as fruits, vegetables, and whole grains can either be present naturally or they may be fortified with additional nutrients and ingredients. Probiotic yogurts, high-fiber cereals, and omega-3-fortified eggs are good examples of functional foods. Fermented vegetables also are considered functional foods due to the presence of probiotics [15,16].

The objective of this research was to manufacture pickled kohlrabi samples in order to widen product diversity and improve the functional properties of kohlrabi. It was also aimed at optimizing and evaluating the effects of various processing factors on the chemical and microbiological properties of fermented kohlrabi by response surface methodology. In this current work, the spontaneous fermentation of kohlrabi was achieved with the target of enhancing the functionality of kohlrabi and the production of a new product by optimizing fermentation conditions.

## 2. Materials and Methods

Pale green kohlrabi used in the fermentation process was obtained from a local agricultural producer (Serpincik, Sivas, Turkiye). All other reagents and solvents are of analytical or chromatographical grade and were provided by Sigma-Aldrich (Germany).

### 2.1. Spontaneous Fermentation of Kohlrabi

A Box Behnken experimental design was constructed to measure the effects of boiling time (0–8 min.), vinegar ratio (0–50% *v:v*), and salt content (2–8% *w:v*) on chemical and microbiological properties of fermented kohlrabi (Table 1); as a result, 15 different fermentation media were prepared. A portion of approximately 500 g of peeled and sliced (2 × 2 cm) kohlrabi samples was steam cooked (Philips, USA SCF870-10). By observing the steam output from the device, the boiling times given in Table 1 were started. Boiled and fresh kohlrabi samples were mixed with NaCl, grape vinegar, and drinking water at the specific ratios given in the design table (Table 1). Additionally, each jar contained a piece of bay leaf and two small cloves of garlic to add flavor and aroma. Lastly, two slices of lemon were put on top of all the ingredients in the jar and the lid was closed. Fermentation was carried out at 25 °C for 28 days.

**Table 1.** Chemical and microbiological properties of fermented kohlrabi samples.

Experiment No	Boiling Time (min.)	Vinegar Concentration (%)	Salt Concentration (%)	Sample Code	pH	Salt Content (%)	Total Acidity (%)	TPC (mg Gallic Acid/100 g)	TAC ( $\mu$ mol Trolox/100 g)	TMYC (log CFU/mL)	TBC (log CFU/mL)	Total LAB (log CFU/mL)
1	4	0	8	K408	3.84	5.00	0.85	11.57	42.39	6.20	6.13	6.07
2	4	0	2	K402	3.16	1.65	1.67	16.00	47.28	7.98	7.28	8.26
3	4	25	5	CP1	3.14	3.43	1.31	26.91	46.47	6.73	6.82	6.39
4	4	25	5	CP2	3.23	3.47	1.42	24.82	43.60	5.73	6.13	6.07
5	0	50	5	K025	3.18	3.17	1.49	31.52	47.33	6.92	7.19	7.40
6	0	25	2	K012	3.19	1.64	1.43	39.88	75.75	7.19	7.08	7.09
7	0	0	5	K005	3.08	3.89	1.27	38.25	65.34	7.09	6.83	7.83
8	0	25	8	K018	3.11	5.03	1.13	35.98	56.56	6.42	7.17	6.62
9	8	50	5	K825	3.14	3.77	1.39	4.71	11.91	6.42	6.70	6.53
10	4	25	5	CP3	3.30	3.49	1.42	5.15	45.82	6.79	6.84	7.86
11	8	0	5	K805	3.08	3.12	1.28	3.83	17.48	7.09	6.83	7.83
12	8	25	8	K815	3.80	5.57	1.30	2.75	15.30	6.42	6.42	6.25
13	4	50	8	K428	3.46	5.40	1.12	7.57	46.47	5.26	6.19	6.20
14	8	25	2	K812	3.16	1.82	1.28	4.35	16.16	6.09	6.19	8.26
15	4	50	2	K422	3.16	1.61	1.65	5.30	44.86	6.09	6.19	8.26

Standard deviations (SD):  $SD_{TMYC} = 0.60$ ;  $SD_{TBC} = 0.41$ ;  $SD_{LAB} = 0.95$ ;  $SD_{pH} = 0.08$ ;  $SD_{SC} = 0.60$ ;  $SD_{TA} = 0.06$ ;  $SD_{TPC} = 12.00$ ;  $SD_{TAC} = 1.50$ . (SD for each measurement was calculated from three replicates of central points in the experimental design). CP = central point.

## 2.2. Chemical Property Analyses

### 2.2.1. Determination of Total Phenolic Content

The total phenolic contents (TPC) of fermented kohlrabi samples were determined with the Folin–Ciocalteu assay [17]. The absorbances of the samples were measured at 765 nm with a UV spectrophotometer (Optima, SP3000, Japan). The total phenol content of the samples was calculated using the gallic acid standard curve and the results were expressed in milligrams of gallic acid equivalent (GAE) per 100 g samples.

### 2.2.2. Determination of Total Antioxidant

The total antioxidant capacity (TAC) of fermented kohlrabi samples was measured by DPPH radical scavenging method [18]. The analyses were performed twice. TAC was expressed as  $\mu\text{mol Trolox}/100\text{ g}$  per g sample.

### 2.2.3. Physicochemical Analyses

Titration with a 0.1 N silver nitrate solution was performed to determine the percentages of the salt content of fermented samples [19]. The pH values of fermented kohlrabi samples were measured with a pH meter (Hanna HI2211, Germany). The total acidity of the samples was evaluated by titration with 0.1 N NaOH solution and calculated as a percentage of lactic acid [20].

## 2.3. Microbiological Property Analyses

One mL of the brine from fermented kohlrabi samples was mixed with 9 mL of 0.85% NaCl solution. Subsequently, the mixture was diluted serially with NaCl solution to such a level that colonies could be counted. To measure the total yeast and mold (TYMC) for mesophilic (TBC) and total lactic acid bacteria (LAB) counts of samples, 0.1 mL of each dilution was added to plates containing potato dextrose agar (PDA), plate count agar (PCA), and De Man, Rogosa and Sharpe agar (MRS), respectively [21]. After 2 days of cultivation at 37 °C for TMB and total LAB and 5 days at 28 °C for TYM, counting was performed. The microbial results were expressed as CFU (colony forming unit) per mL sample.

## 2.4. Statistical Analyses

The data were analyzed by the ANOVA in order to investigate the effects of salt content, boiling time, and vinegar concentration, as well as their interaction ( $p < 0.05$ ) on chemical and microbiological properties of the fermented kohlrabi samples and to optimize fermentation conditions of kohlrabi (Minitab 19 software, UK). Pearson correlation coefficients ( $r$ ) for relationships between various responses were calculated (Minitab 19 software, UK). Moreover, the principal component analysis (PCA) was also used (SIMCA 14.1, MKS Umetrics, Umea, Sweden) to investigate the effects of chosen factors on the lactic fermentation of kohlrabi and also for the classification of fermented samples. The  $t$ -test was used to determine whether there was a statistical difference between the responses obtained after and before optimization (Minitab 19 software, UK).

## 3. Results and Discussions

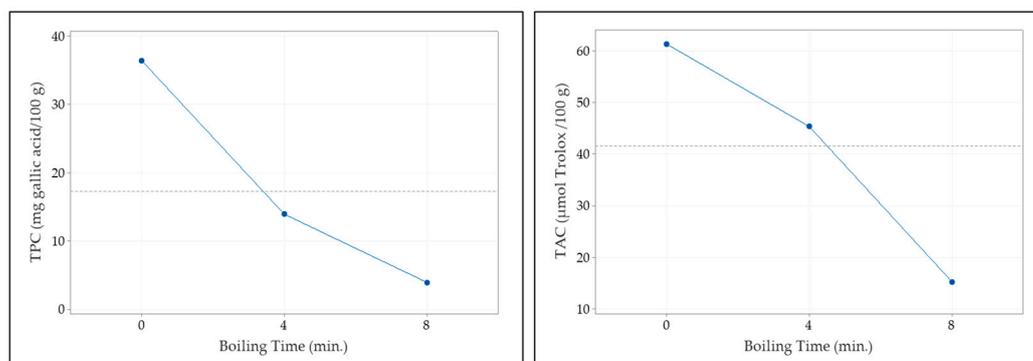
### 3.1. Chemical Properties of Fermented Kohlrabi

The TPC of the fermented kohlrabi samples is given in Table 1. The TPC of fermented samples ranged from 2.75 to 39.88 mg gallic acid/100 g sample. The TPC of fermented kohlrabi samples decreased by proceeding with boiling time. In particular, the samples that fermented without the boiling step had higher TPC values compared to all other samples. This result could be associated with the damage of heat treatments on the phenolic compounds of kohlrabi (Table 1). It was determined that the application of different heating techniques on kohlrabi (conventional, microwave, and ohmic heating) induced some declines in TPC values [22]. The TPC results of fermented kohlrabi samples corresponded to a prior study in which the phenolic contents and antioxidant activity of 25 different *Brassicaceae* family vegetables were extensively investigated [23]. The statistical

model constructed for TPC was significant at the 95% confidence interval ( $R^2 = 0.89$  and  $R_{adj}^2 = 0.69$ ) and the boiling time was only important for the TPC model of fermented kohlrabi samples (Table 2). The main effect plot also revealed that boiling time had a remarkable impact on the TPC of fermented kohlrabi samples. The lowest TPC value was obtained when the boiling time reached 8 min before fermentation (Figure 1).

**Table 2.** ANOVA table of chemical and microbiological parameters for fermented kohlrabi samples.

	pH	Salt Content (%)	Total Acidity (%)	TPC (mg Gallic Acid/100 g)	TAC ( $\mu\text{mol Trolox}/100\text{ g}$ )	TMYC (log CFU/mL)	TBC (log CFU/mL)	LAB (log CFU/mL)
<i>p</i> -value of model	0.13	0.00	0.31	0.05	0.00	0.40	0.58	0.38
<i>p</i> -value of lack of fit	0.14	1.00	0.07	0.99	0.04	0.52	0.47	0.75
$R^2$	0.84	1.00	0.74	0.89	0.97	0.70	0.62	0.71
$R_{adj}^2$	0.55	1.00	0.28	0.69	0.91	0.17	0.00	0.20
$Q^2$	0.00	0.92	0.00	0.66	0.50	0.00	0.00	0.00
<b><i>p</i>-value of factors</b>								
Boiling Time (BT)	0.24	0.27	0.87	0.00	0.00	0.39	0.15	0.97
Vinegar Concentration (VC)	0.65	0.54	0.30	0.40	0.23	0.09	0.54	0.50
Salt Concentration (SC)	0.02	0.00	0.02	0.74	0.20	0.14	0.53	0.03
<b><i>p</i>-value of interactions</b>								
BT*BT	0.31	0.47	0.74	0.23	0.06	0.38	0.27	0.57
VC*VC	0.94	0.69	0.97	0.27	0.39	0.64	0.96	0.38
SC*SC	0.08	0.90	0.49	0.39	0.40	0.57	0.51	0.92
BT*VC	0.91	0.01	0.76	0.65	0.32	0.70	0.60	0.60
BT*SC	0.08	0.31	0.42	0.89	0.16	0.41	0.88	0.37
VC*SC	0.30	0.22	0.44	0.69	0.59	0.47	0.25	0.94



**Figure 1.** The main effect plots of boiling time on TPC and TAC of fermented kohlrabi.

The TAC values of samples are presented in Table 1. The TAC values of samples gradually decreased as boiling duration progressed (Table 1) which was also observed in the TPC of fermented kohlrabi samples. Previous research demonstrated a relationship between the TAC and TPC of fermented kohlrabi. It was discovered that the higher the TPC content of kohlrabi, the higher the TAC content [24]. Therefore, a Pearson correlation was established between the TPC and TAC data of samples and the ‘r’ value was calculated as 0.80 (Table 3). The Pearson correlation value confirmed the fact that the TPC and TAC values are very highly correlated. According to the ANOVA table, the created model for TAC values of fermented kohlrabi samples was ( $R^2 = 0.97$  and  $R_{adj}^2 = 0.91$ ) significant ( $p < 0.05$ ). Boiling time and its square interaction were the prominent factors affecting the TAC of fermented kohlrabi samples (Table 2). Furthermore, the main effect plot indicated that increasing boiling time caused reductions of the TAC of fermented samples (Figure 1).

**Table 3.** The Pearson correlation coefficient values of responses.

	pH	TA (%)	SC (%)	TPC (mg Gallic Acid/100 g)	TAC ( $\mu$ mol Trolox/100 g)	TMYC (log CFU/mL)	TBC (log CFU/mL)
pH							
TA%	0.6						
SC%	−0.37	−0.72					
TPC (mg gallic acid/100 g)	−0.22	0.02	−0.07				
TAC ( $\mu$ mol Trolox/100 g)	−0.36	0.07	−0.14	0.80			
TMYC (log CFU/mL)	−0.49	0.44	−0.46	0.32	0.24		
TBC (log CFU/mL)	−0.54	0.35	−0.22	0.54	0.38	0.82	
LAB (log CFU/mL)	−0.54	0.57	−0.73	−0.11	0.03	0.52	0.30

The salt percentages of samples fluctuated regardless of boiling time and vinegar concentration. Furthermore, the higher the NaCl concentration in the fermentation media, the higher the SC% of fermented kohlrabi (Table 1). This result could be associated with the NaCl absorption of fermented kohlrabi samples. The effect of salt concentration on the fermentation of kohlrabi was examined in previous work, and it was suggested that the fermentation of kohlrabi should be carried out using a 3.5% NaCl solution [25]. However, in this current study, the SC% of kohlrabi samples was in the range of 1.61–5.57%. Since most of the fermented kohlrabi samples consist of lower salt content, they could be preferred by individuals with high blood pressure. The ANOVA table indicated that the model for SC% of fermented kohlrabi samples was important ( $R^2 = 1$  and  $R_{adj}^2 = 1$ ). It was found that the salt concentration of fermentation media, as well as the interaction of vinegar concentration and boiling time, had substantial effects on SC percentages of fermented kohlrabi samples (Table 2).

The pH of the samples changed in the range of 3.08–3.84 and the total acidity values of the samples varied between 0.85 and 1.67% (Table 1). The previous study related to the lactic acid fermentation of kohlrabi demonstrated that the organic acids produced by LAB cause a decrease in the pH values [25]. The pH and total acidity percentages of our study were also compatible with this work. Moreover, a Pearson correlation between the total acidity and pH values of the samples was established. Since the correlation coefficient ‘r’ value was calculated as −0.54, the pH and total acidity values of fermented samples had a negative and medium association (Table 3). The Pearson correlation explained that pH values decreased as total acidity increased (Table 1). The models developed for physicochemical properties of fermented kohlrabi samples including pH and total acidity were found to be insignificant ( $p > 0.05$ ) (Table 2).

### 3.2. Microbiological Properties of Fermented Kohlrabi

At the end of 28 days of fermentation, the brines of fermented kohlrabi samples were utilized in microbiological analyses, including TMYC, TBC, and total LAB count. The samples ranged from 5.26–7.79 CFU/mL for TMYC, 6.13–7.27 CFU/mL for TBC, and 6.07–7.83 CFU/mL for LAB (Table 1). ANOVA table showed that the models built for TMYC, TBC, and total LAB are statistically insignificant ( $p > 0.05$ ) (Table 2).

A similar study employed a solution of 2.5% salt and 15% crystal sugar to produce naturally fermented cucumbers [26]. It had been stated that the increase in both TBC and total LAB counts was significant and time dependent ( $p < 0.05$ ). While the TBC of fermented cucumber samples exceeded 8 log CFU/mL after 16 days of fermentation, the LAB counts reached 11 log CFU/mL.

Although some yeasts produce pectinase, which softens fermented vegetables through depectinization, yeasts are regarded to be crucial to the formation of flavor in fermented vegetables [27,28]. The correlation between TMYC and general acceptance was significant with an ‘r’ value of 0.53 (Table 3). Thus, it can be presumed that the quantity of TMYC in fermented kohlrabi samples had an effect on general acceptance.

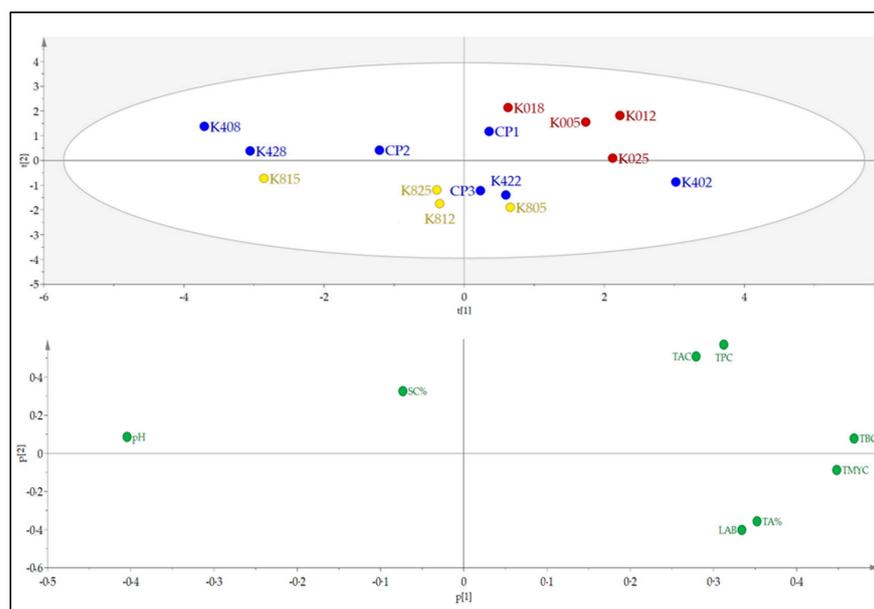
In a previous study, after four months, the number of yeasts in sauerkraut juice fermented with 1.2% NaCl was much larger than in sauerkraut juice fermented with 0.5% NaCl, indicating that 1.2% NaCl was more favorable to yeast growth [29]. In our research,

2–8% salt was added to the fermentation media before initiating the fermentation of kohlrabi. Although the correlation between salt content and TMYC was found to be insignificant, the correlation between salt concentration and LAB counts was shown to be quite notable ( $r = -0.70$ ) ( $p < 0.05$ ) (Table 3). There was a strong and negative correlation between the SC % of fermented kohlrabi samples and the quantity of total LAB present in the fermentation media.

In this current work, total counts of LAB of fermented Kohlrabi samples were elaborated. The total LAB counts (6.07–8.26 log cfu/mL) of fermented kohlrabi samples exhibited their potential as a functional food. The LAB present in pickled foods has been associated with a variety of probiotic (health-related) characteristics, including improved digestion, lower blood cholesterol levels, cancer suppression, and an increase in natural resistance to infectious disease in the gastrointestinal tract [30]. Naturally fermented products are well-known to be a type of lacto-fermentation in which substantial amounts of lactic and acetic acids accumulate as a result of LAB growth by causing the pH to drop. These outcomes not only provide desirable organoleptic characteristics but also ensure both safety and stability of the end product by destroying pathogen microorganisms [31].

### 3.3. Principal Component Analysis of Fermented Kohlrabi Samples

In order to better explain the fermentation process of kohlrabi and to classify the samples, a principal component analysis (PCA) was applied to the whole data. The number of components of the PCA model was 8 and  $R^2 = 0.99$ ,  $Q^2 = 0.62$ . The samples were colored with respect to boiling time (Figure 2). Since the scatter plot is examined, it was observed that the kohlrabi samples were grouped together according to the boiling time (Figure 2). The samples fermented without boiling step were differentiated from other samples by placing them in the upper right part of the plot. The samples boiled for 8 min before fermentation were clustered in the bottom left part of the ellipse. The samples boiled for 4 min and took place around the center of the score plot. The non-boiled fermented kohlrabi samples were separated from others in terms of TPC and TAC (Figure 2). It has been previously discussed that the samples without boiling stage had higher TPC and TAC values compared to the other samples (Table 1).



**Figure 2.** Score plot (coloring is done with respect to boiling time; red: 0 min, blue: 4 min, yellow: 8 min) and loading plot obtained from principal component analysis for chemical and microbiological parameters of fermented kohlrabi samples.

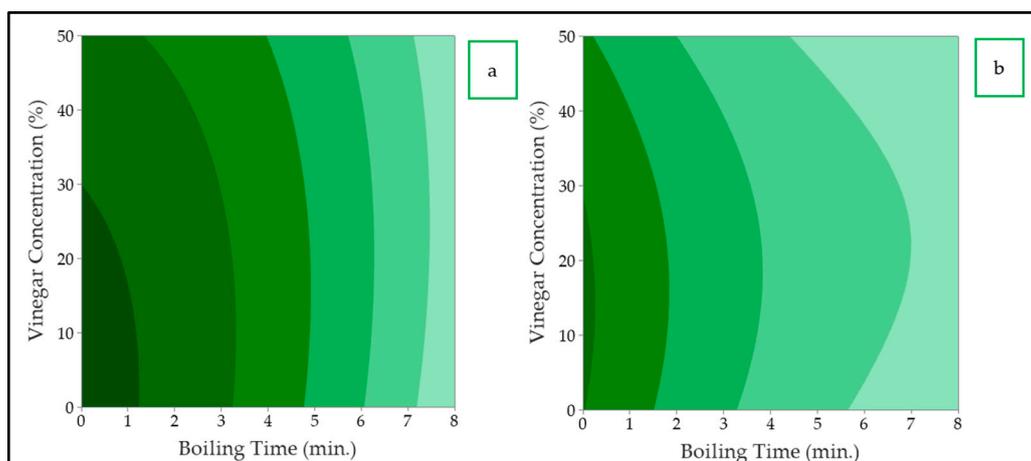
### 3.4. Optimization of Spontaneous Fermentation of Kohlrabi

Since one of the objectives of this work is to produce a new fermented product of kohlrabi, an optimization process was performed by considering the TPC and TAC values of the samples. The response optimization suggested achieving a fermentation process without boiling stage and 44.12% vinegar and 2.07% salt concentrations to reach maximum values of TPC and TAC (obtained as TPC = 39.88 mg gallic acid/100 g and TAC = 75.75  $\mu\text{mol}$  Trolox/100 g). Therefore, a new fermentation media was generated with fresh kohlrabi in a brine containing 44.12% vinegar, 2.07% NaCl, and 53.81% drinking water. The fermentation of kohlrabi proceeded again at 25 °C for 28 days. Similar results were obtained (TPC = 38.79 mg gallic acid/100 g and TAC = 73.82  $\mu\text{mol}$  Trolox/100 g) after the natural fermentation of kohlrabi. Moreover, the *t*-test also confirmed that there was no significant difference between predicted and actual data of TPC and TAC of fermented kohlrabi ( $p > 0.05$ ). The regression equations of TPC and TAC of fermented kohlrabi samples were calculated as:

$$\text{TPC (mg gallic acid/100 g)} = 35.0 - 7.59 \text{ Boiling Time (min)} + 0.110 \text{ Vinegar Concentration (\%)} + 3.20 \text{ Salt Concentration (\%)} + 0.352 \text{ Boiling Time (min)} \times \text{Boiling Time (min)} - 0.00801 \text{ Vinegar Concentration (\%)} \times \text{Vinegar Concentration (\%)} - 0.427 \text{ Salt Concentration (\%)} \times \text{Salt Concentration (\%)} + 0.0190 \text{ Boiling Time (min)} \times \text{Vinegar Concentration (\%)} + 0.048 \text{ Boiling Time (min)} \times \text{Salt Concentration (\%)} + 0.0223 \text{ Vinegar Concentration (\%)} \times \text{Salt Concentration (\%)}$$

$$\text{TAC (\mu mol Trolox/100 g)} = 87.1 - 4.92 \text{ Boiling Time (min)} - 0.123 \text{ Vinegar Concentration (\%)} - 6.03 \text{ Salt Concentration (\%)} - 0.440 \text{ Boiling Time (min)} \times \text{Boiling Time (min)} - 0.00438 \text{ Vinegar Concentration (\%)} \times \text{Vinegar Concentration (\%)} + 0.299 \text{ Salt Concentration (\%)} \times \text{Salt Concentration (\%)} + 0.0311 \text{ Boiling Time (min)} \times \text{Vinegar Concentration (\%)} + 0.382 \text{ Boiling Time (min)} \times \text{Salt Concentration (\%)} + 0.0217 \text{ Vinegar Concentration (\%)} \times \text{Salt Concentration (\%)}$$

The response surface contour plots were shown in Figure 3a,b. These graphics illustrate the effects of vinegar concentration and boiling time on the TPC and TAC values of fermented kohlrabi at a constant salt ratio of 5%. The dark green regions point to the optimal fermentation conditions. The vinegar ratio should be between 0 and 30% for the production of fermented kohlrabi with the highest antioxidant activity, and the boiling period is limited to one minute (Figure 3a). Moreover, fermented kohlrabi samples with the highest phenolic content could be performed with the vinegar ratio ranging from 0–30% without the boiling stage (Figure 3b).



**Figure 3.** Contour plot of effect of boiling time and vinegar concentration on responses: (a) TAC of fermented kohlrabi samples; (b) TPC of fermented kohlrabi samples.

#### 4. Conclusions

A new fermented product was manufactured by spontaneous fermentation of Kohlrabi, and some chemical and microbiological properties of this product were determined. It was found that the end product produced under optimal conditions had higher LAB counts and TPC, which is quite promising for food functionality. Since most of the fermented kohlrabi samples have low salt content, they could be recommended for the diets of people who prefer limited amounts of salt in their daily diet. Both ANOVA and PCA results confirmed that boiling time was the most important factor that affects the fermentation process of kohlrabi. The microbiological properties of fermented kohlrabi were reasonable. The total quantity of LAB present in fermented samples was satisfactory, indicating the potency of the end product as a functional food. The optimal fermentation conditions of kohlrabi were established to be 44.12% vinegar and 2.07% salt concentrations. It was also suggested that the fermentation could be accomplished by fresh kohlrabi at 25 °C for 28 days in order to attain maximum TPC and TAC values. The changes in chemical and microbiological properties of fermented kohlrabi samples packaged with different techniques throughout the storage period could be examined in a future study.

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