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Effect of *Arctium Lappa* Extract Inoculated with *Lactobacillus Plantarum* On Fermented Salted Cabbage

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ABSTRACT

Introduction: The present study aimed to assess the effect of *Arctium lappa* extract inoculated with *Lactobacillus plantarum* probiotic bacteria on fermented salted cabbage. In addition, the microbial, chemical, and sensory properties and survival experiment of the bacteria were evaluated during storage.

Methods: *Lactobacillus plantarum* was used as a probiotic species in fermented cabbage. The effects of various levels of salt (1% and 1.5%) and Burdock extract (2 and 4 mg/ml) were evaluated and compared with control samples (without the dandruff extract) with the inoculation of *Lactobacillus plantarum* probiotic bacteria as the starter.

Results: Increased concentration of the *Arctium lappa* extract and minimum concentration of salts led to the significant reduction of yeasts and aerobic mesophyll (1.5% salt and 4 mg/ml of the herbal extract). On the other hand, decreased concentration of salt and increased concentration of the *Arctium lappa* extract led to the increased viability of *Lactobacillus*, and the maximum survival of *Lactobacillus plantarum* was observed in the treatment with 1% salt and 4 mg/ml of the extract on day 21.

Conclusion: According to the results, using *Lactobacillus plantarum* as the starter, along with salt and the *Arctium lappa* extract, could improve the microbial control of the fermentation process, while increasing the production of lactic acid and improving the acidity of the saline of salty cabbage.

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Introduction

Today, various chemical additives are used in different industries, which are known to adversely affect human health and cause severe public health issues. The production of additives to preserve the desirable features of food products with the least adverse health effects has attracted the attention of researchers. In this regard, lactic acid bacteria are considered to be a viable option. In addition to their beneficial health effects on humans, these bacteria have been reported to enhance the shelf life of food products by acting as preservatives (1, 2). Moreover, probiotic bacteria have other therapeutic effects in addition to the improvement of gastrointestinal disorders; such examples are the lowering of blood cholesterol, anticancer properties, improvement of the immune function, reducing the side-effects of antibiotics, increasing the absorption of vitamins and minerals in the gastrointestinal tract, and decreasing lactose intolerance (3, 4). The survival of probiotic bacteria is influenced by several factors, such as strain properties, food matrices, and temperature (5). Tolerance of environmental stress varies in different bacterial species and strains. The growth and optimal sensory composition of these bacteria depend on the public starter, which must survive during the fermentation process and exhibit metabolic activity. By definition, probiotics are referred to as living bacteria, and the assurance of the quality of probiotic products depends on the identification of living cells (6).

Lactic acid bacteria could be used to suppress the growth of pathogenic bacteria, improve the taste of food products, reduce energy consumption compared to other food preservation methods, and facilitate food preparation and maintenance without the need for cooling systems (7). Since fruits and vegetables inherently contain beneficial nutrients (e.g., minerals, vitamins, dietary fibers, and antioxidants) without the allergens found in dairy products, they are

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considered to be effective in the development of fermented and probiotic food products (8, 9).

Baba-adam (*Arctium lappa*) is a grassy plant belonging to the chicory strain. *Arctium lappa* is an indigenous plant originating from warm Asian regions, which has large green leaves. The chemical composition of *Arctium lappa* roots includes glycosides, flavonoids, tannins, volatile oil, and inulin. The seeds of this plant also contain a specific type of yellow oil with the bitter taste of linoleic acid and oleic acid. Use of *Arctium lappa* extracts has attracted attention owing to its remarkable antioxidant properties and synergistic effects with probiotic lactic acid bacteria (10).

Sauerkraut is a fermented cabbage product, which is considered to be a supplementary food product owing to its nutritional properties. The high nutritional value of this product and its better digestibility compared to raw cabbage have encouraged its production within the past two decades. Lactic acid is the dominant bacterium in the preparation of sauerkraut, which contains *Leuconostoc mesenteroides*, *Leuconostoc falks*, and *Lactobacillus plantarum* (2, 11).

Lactobacillus plantarum is an important strain of lactic bacteria, which has been detected in the brine of fermentation products. This active bacterium produces the required acidic lacquer for the protection of these products. In addition, use of appropriate *Lactobacillus plantarum* cultures and herbal extracts could increase the production of lactic acid and improve the microbial control of the process (12).

Several studies have indicated that fruits and vegetables could be a proper basis for the production of super supplemental probiotic products. In 2006, the analysis of the probiotics in olive demonstrated that using the probiotic strain of *Lactobacillus paracasei* as the primer culture in olive fermentation caused the probiotic strain to become predominant in fermentative olives through reducing the salt concentration from 8% to 4% and increasing the storage temperature from 4°C to 25°C (13). On the other hand, evaluation of the effects of inulin extracted from *Arctium lappa in-situ* confirmed the presence of *Arctium lappa* inulin, while the concentrations of *Bifidobacterium* and *Lactobacillus* were reported to increase significantly ($P<0.05$). Therefore, it could be concluded that the beneficial health effects were resulted from *Arctium lappa* inulin (14).

The present study aimed to improve the sensory and microbial properties of a final product using the *Arctium lappa* extract and probiotic bacteria. Furthermore, the effects of various concentrations of salt (1% and 1.5%) and *Arctium lappa* extract (2 and 4 mg/ml), along with the inoculation of *Lactobacillus plantarum* probiotic bacteria as the starter for the production of probiotic salted cabbage were evaluated. The microbial, chemical, and sensory properties (e.g., acidity, color, taste, and tissue) of salted cabbage and the survival of the probiotic bacteria were investigated during the storage of salted cabbage.

Materials and Methods

The culture media of YGC, plate count agar (PCA), and De Man, Rogosa and Sharpe agar (MRS) were purchased from Merck Company (Merck, Germany). Double distilled water was used in all the experiments. The roots and leaves of *Arctium lappa* were obtained from the Medicinal Plants Department of the Ministry of Tehran Agricultural Jihad Institute, Iran. Additionally, *Lactobacillus* ATCC 6935 was provided by the Microbiology Laboratory of Tehran University, Iran. White leaves of cabbage were purchased from the suburbs of Tehran.

Preparation of Arctium lappa and Herbal Extract

The roots of *Arctium lappa* were collected from a field of medicinal plants at Tehran Agricultural Jihad Institute and placed in a freezer bag until processing. The plant samples were powdered using a mixer and poured into a container. Afterwards, the powder was pre-wetted with the desired solvent, and 96% ethanol was added to the container in order to cover the entire samples, which were locked and placed in a shaker for 72 hours. Following that, the samples were filtered, and the solvent was completely removed from the extract using a rotary device with evaporating aid.

Preparation of Salt Brine

In order to prepare the salt water, sodium chloride and distilled water were used. Afterwards, the brine was autoclaved in falcon tubes with a constant volume ratio, and the cabbage samples were added to a constant weight ratio in sterilized conditions.

Preparation of Probiotic Bacterial Suspension

The strains of *Lactobacillus plantarum* were used in the present study. The probiotic bacterial suspension was activated by incubation at the temperature of 37°C for 48 hours before it was added to the fermented Sauerkraut. Initially, microbial living cells were separated through biomass centrifugation (Haniel, South Korea) at 5,000 grams for 15 minutes at the temperature of 4°C. Afterwards, each of the activated probiotic strains in the main volume of salted water was added to the shredded cabbage samples. The concentrations of salt (1% and 1.5%) and *Arctium lappa* roots (2 and 4 mg/ml) were applied for 21 days in the mentioned conditions (15).

Sampling

The fermentation of Sauerkraut was performed in a small glass container using 1 and 1.5 grams of salt and 2 and 4 mg/ml of *Arctium lappa* root extract with 69.5 grams of chopped cabbage using the auto-fermentation method. *Lactobacillus plantarum* cells were activated and inoculated into the salted cabbage, and the fermentation process was performed for one week at the temperature of 24°C. Following that, the samples were preserved at room temperature for 21 days. Table 1 shows the compounds of the prepared samples.

Table 1. Formulation of Sauerkraut Production

Samples	Compounds	
	Salt (g)	Root Extract
Control	2	0
T1	2	2
T2	2	4
T3	1.5	2
T4	1.5	4

Microbial Analysis during Fermentation

The microbial analysis in the salted water samples was performed one day after fermentation and on day 21 when the fermentation process was complete. At the next stage, ml of brine solution (1 ml) was added to 9 ml in sterilized conditions, and fractional dilutions were carried out in a diluted solution (16). Following that, 0.01 mM of diluent solution was cultivated on the surface cultivation environment of the MRS agar. Yeast counting was performed in three replicates using a pour plate on the MRS glucose media chloramphenicol agar. The counting was carried out three days after fermentation. Cultivation on the YGC agar containing antibiotic chloramphenicol was performed in order to analyze the mold count in the species of *Saccharomyces*, *Rhodotorula*, *Candida*, *Kluyveromyces*, *Saccharomyces*, and *Aspergillus* (17).

Chemical Measurements

Acidity

At this stage, five milliliters of the Sauerkraut extract was added to distilled water, and titration was performed using sodium chloride (0.1 N), and the results were expressed as the percentage of the lactic acid bacteria (18).

Sensory Evaluation

A panel of semi-trained experts evaluated the sensory properties of the probiotic salted cabbage samples, such as the color, flavor, and general acceptability. The tests in this regard were performed under similar light and temperature conditions. In order to prevent odor interference during the evaluation, some coffee was used before each olfactory experiment. In the multiple comparison tests, the treatments were assigned to the members of the sensory group using specific codes, and the groups were asked to compare the samples and respond to the questions in the prepared questionnaire, in which the scores of four and five denoted good and excellent treatment, respectively, while average treatment was scored three, and poor and extremely poor treatments were scored one and two, respectively (19).

Statistical Analysis

Data analysis was performed in SPSS version 18. The quantitative data were analyzed using chemical tests were applied after data normalization and control analysis of the variance. In addition, the comparison of means was carried out at the significance level of 5% using Duncan's test (7).

Results

Lactobacillus plantarum Survival

The trend of changes was evaluated in the bacterial count (*Lactobacillus plantarum*) in the samples containing various concentrations of the herbal extract and control samples on day 21.

The maximum bacterial count was observed on day seven of the experiment (completion of the fermentation process) (Figure 1).

On day 21 of preservation, treatments two and four showed higher percentage of the *Arctium lappa* extract and larger number of the surviving *Lactobacillus*. In the control samples, the number of the surviving bacteria decreased significantly ($P<0.05$). At various concentrations of the herbal extract, the survival of the bacteria had a significant difference compared to the control samples. In most of the samples, the addition of the herbal extract was observed to improve the survival of the bacteria.

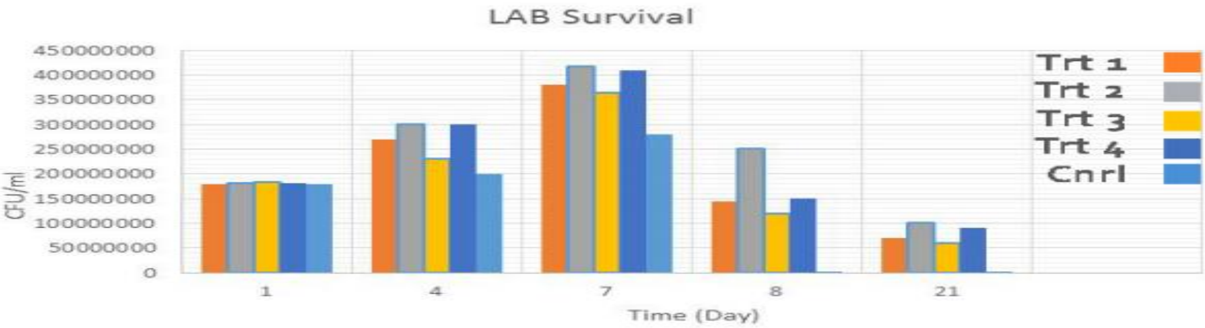

Figure 1. *Lactobacillus plantarum* Survival in Fermented Probiotic Cabbage with and without Herbal Extract at Various Times

Yeast Count

As is depicted in Figure 2, no significant difference was observed between the samples of probiotic Sauerkraut in terms of yeast count on the first day of storage. On the first day, the population of yeasts in all the treatments was lowest during fermentation and storage, while these levels increased significantly in all the samples. On the other hand, the yeast count on the YGC culture medium was considered statistically significant in each treatment on a specific fermentation day ($P<0.05$).

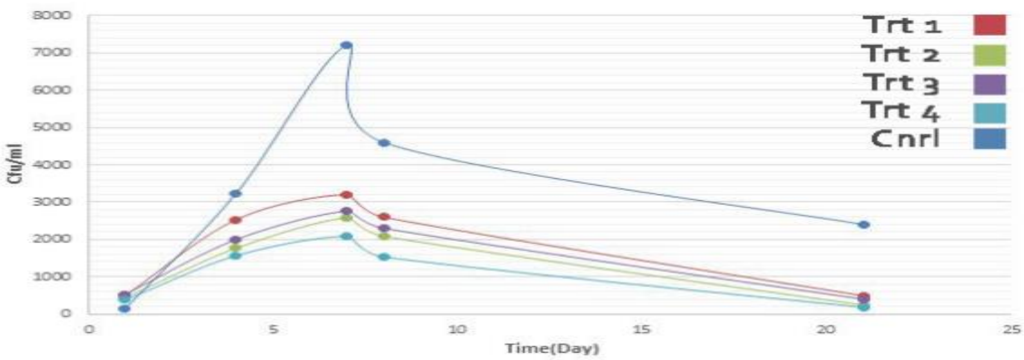

Figure 2. Yeast Count in Probiotic Fermented Cabbage with and without Herbal Extract at Various Times

During days 1-7 of fermentation, the yeast population in all the treatments increased significantly, and this trend was followed by a gradual reduction. On day seven, the yeast population in the samples with 4 mg/ml of the *Arctium lappa* extract was observed to be lower compared to the other samples. In general, the samples without the inoculum and extract of *Arctium lappa* (control) had highest yeast count during fermentation.

Aerobic Mesophilic Bacteria Count

As is shown in Figure 3, no significant difference was observed between the specimens of probiotic Sauerkraut in terms of the probiotic Sauerkraut count on the first day. According to the statistical analysis, the aerobic mesophilic bacteria were significantly different during fermentation. On the first day, the population of aerobic mesophilic bacteria in all the treatments was lowest, while on day seven, the treatments containing 4 mg/ml of the herbal extract had minimum levels of mesophilic bacteria compared to the other treatments.

The largest population of the mesophilic bacteria was observed on day four in all the treatments. In general, a significant difference was denoted in this regard on day eight due to the pasteurization process and noticeable effect of the herbal extract concentration and salt on the reduction of bacteria. In addition, the bacterial population decreased on day 21, and the optimal treatment for the mesophilic bacterial population was observed in the treatments with 1.5% of salt with 4 mg/ml of the herbal extract and 1% of salt with 4 mg/ml of the herbal extract.

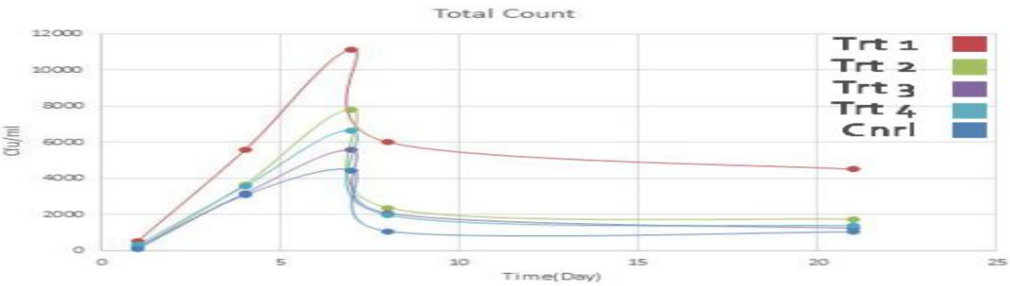

Figure 3. Aerobic Mesophilic Bacterial Count in Fermentative Probiotic Cabbage with Herbal Extract and without Herbal Extract at Various Times

Acidity

Figure 4 shows the changes in the acidity of the probiotic Sauerkraut based on the significance of various times in different treatments.

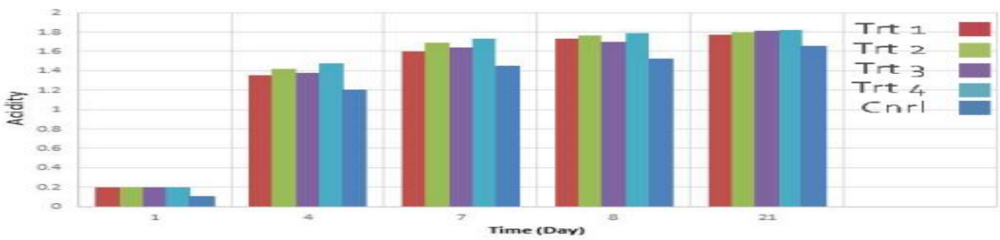

Figure 4. Acidity Changes in Probiotic Fermented Cabbage with and without Herbal Extract at Various Times

According to the obtained results, the acidity values of the probiotic cabbage samples increased significantly during storage, with the maximum acidity denoted in the treatment with 1.5% of salt and 4% of the herbal extract and minimum acidity observed in the control samples. At all the concentrations of the herbal extract, the acidity of the samples increased with the increased shelf life. In fact, the growth of the probiotic bacteria increased acidity due to the production of organic acids.

Sensory Analysis

As is depicted in Figure 5, samples two and four had the highest score compared to the other samples on the third day, while no significant difference was observed between treatments one and three ($P<0.05$). On the other hand, the control sample had the lowest score compared to the other samples. On all the days of the experiment, treatments two and four had the highest score in terms of total acceptance, while the control sample was reported to be non-consumable on day 21. According to the information in Table 2, the control treatment had the lowest score, while treatment four (1.5% of salt and 4 mg/ml of the herbal extract) had the highest score during the experiment compared to the other treatments.

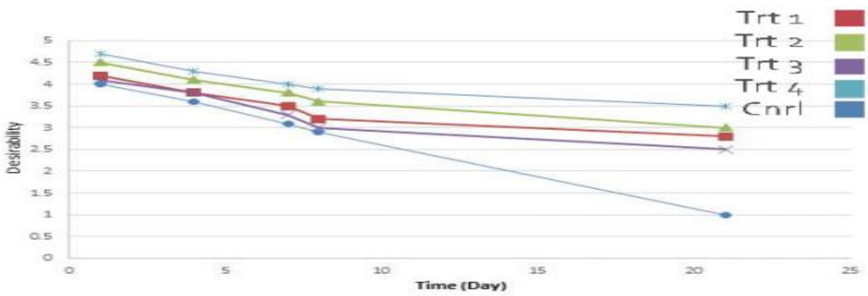

Figure 5. Total Acceptance Index of Probiotic Fermented Cabbage with and without Herbal Extract at Various Times

Table 2. Sensory Analysis Results

	Desirability	Odor	Taste	Color
Trt 1	3.72±0.89[a]	3.78±1.02[a]	3.77±0.97[a]	3.50±0.76[a]
Trt 2	3.80±0.19[a]	3.92±0.63[a]	3.91±0.61[a]	3.78±0.37[a]
Trt 3	3.75±0.32[a]	3.88±0.49[a]	3.93±0.48[a]	3.87±0.72[a]
Trt 4	3.92±0.54[a]	4.01±0.43[a]	4.02±0.49[a]	4.00±0.15[a]
Trt 5	2.32±0.27[a]	2.90±0.52[a]	2.22±0.56[a]	2.78±1.06[a]

[a]*Means with different letters indicate significant differences (P≤0.05)*

Discussion

In the present study, probiotic bacteria were selected due to their high resistance to acidic conditions. These bacteria are able to metabolize sugar and produce small concentrations of organic acid, thereby resulting in increased acidity (20). According to the findings of the current research, pasteurization on day eight of the experiment resulted in the more significant effect of the *Arctium lappa* extract on the samples. In addition, the salt and herbal extract concentrations had significant effects on the survival of *Lactobacillus plantarum* (21). *Lactobasilus plantarum* could survive until the end of the process, and this finding is consistent with the results of the previous studies in this regard (15, 22). On the other hand, the samples containing the *Arctium lappa* extract preserved more *Lactobacillus plantarum* in the product, which could be due to the removal of other microbes to achieve antibacterial effects (23).

With regard to the yeast count, it could be stated that along with the bacteria, lactic acid was detected throughout fermentation in the present study, which is consistent with the findings of Quintana (24). The addition of the *Arctium lappa* extract stimulated the metabolic activity of the starter and increased acidity. Furthermore, the *Arctium lappa* extract changed the media to an acidic environment. As a result, the samples had higher acidity due to microbial activity, as well as higher acid metabolites and probiotic bacterial activity during storage (25). In another study in this regard, it has been reported that the addition of a plant containing inulin to probiotic yogurt could increase the production of lactic acid, decrease the fermentation time, and increase acidity (26).

Previous studies have investigated the effects of *Lactobacillus plantarum* on the properties of probiotic dough during refrigerated storage, demonstrating that the acidity of probiotic dough was higher compared to the control samples due to the presence of *Lactobacillus*, which in turn increased acidity (27).

In the present study, the addition of the *Arctium lappa* extract to the fermented cabbage stimulated the premier bacteria, thereby increasing acid production (28). In a similar study, evaluation of the effects of spice and salt on the fermentation of a type of sausage indicated that spice could increase the fermentation rate, and the samples containing 2% and 3% of salt were superior to the other samples in terms of fermentation, texture, color, and taste (19). The findings of the mentioned research also denoted that increasing the concentration of oregano from 0.5 to 8 gr/l induced *Lactobacillus plantarum*, thereby increasing acid production in the samples (29).

Another research in this regard assessed the production of probiotic peach juice using three probiotics, including *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, and *Lactobacillus casei*. According to the findings, the highest acidity during fermentation was induced by *Lactobacillus delbrueckii* (30). Similar results have been reported in case of beetroot juice containing *Lactobacillus acidophilus*, and acidity was higher compared to the control samples (31).

Conclusion

According to the results, probiotic bacteria used carbohydrates for the production of organic acids, thereby increasing acidity. Therefore, it could be concluded that *Lactobacillus plantarum* affected the sensory properties of salty cabbage. The optimal results in this regard were observed in the treatment with 1% of salt and 4 mg/ml of the herbal extract on day 21.

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