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Effect of drying on the survival and performance of probiotics in dried fruits

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ABSTRACT

The purpose of this study was to evaluate the effect of drying on the survival and performance of probiotics in dried fruits. In this study, banana, apple, cucumber and apricot fruits and four probiotic strains including *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Lactobacillus lactis* were studied. The antimicrobial properties of Lactobacillus products before and after drying were investigated on four bacterial pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* using batch fermentation system. Data analysis was analysis using Minitab software and the effect of different variables such as type of bacteria, temperature, drying time and type of fruit on the survival and probiotics function was evaluated using Taguchi experimental design. *Lactobacillus* spp. showed antimicrobial properties against pathogenic bacteria before and after drying. Also, data analysis showed that drying did not threaten the survival of probiotics, the highest growth rate for *Lactobacillus rhamnosus* was observed after 68 hours, indicating that, after drying, probiotics were retained for up to hours and were able to grow up. Finally, the analysis of the data showed that the bacterial species and different temperatures had a significant relationship with the survival of lactobacillus after drying. Therefore, probiotic dried fruits are proposed as a new idea in the food industry.

1. Introduction

Probiotics are vital microorganisms had beneficial health effect on host with different mechanism (Meng et al., 2008). Lactobacillus and bifidobacteria are the most important probiotics and have the characteristics of acid and gallbladder tolerance, organic acid production, hydrogen peroxide and antimicrobial compounds expansion of the use of probiotics has led to the selection of microorganisms with a positive effect on health. Although the mechanism of action of probiotic bacteria is not entirely clear, recent findings indicate that they can interact with the surface layer of microorganisms, bacterial DNA, or in the synthesis of essential metabolites. (Ebel et al., 2014). Since industrial production processes can

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significantly alter the structural and functional properties of microorganisms, the use of probiotics in both clinical and food sectors requires strategies to enhance their performance (Ebel et al., 2014). Thus, in order to maintain the durability and long-term potential of probiotics, biotechnology industry uses the drying process to survive. Several studies have shown that micro dehydrated organisms remain active at room temperature for a long time (Meng et al., 2008; Zayed & Roos, 2004). Although water is a necessary ingredient for life, lactic acid bacteria have a high potential for survival under drought conditions. Today, researchers are looking for new methods to study the effect of drying on probiotics, by various methods such as spraying, freezing and air drying (Meng et al., 2008; Zayed & Roos, 2004; Huang et al., 2017). Today, large application of probiotics has been observed in the food industry. It should be noted, however, that the target probiotics should survive and this does not mean that the probiotics are stable indefinitely (Farazandehnia, 2016). According to the above, the purpose of this study was to evaluate the effect of drying on the survival and performance of probiotics in dried fruits, which could be considered as new ideas in the food industry for the production of dried fruit containing probiotics.

2. Materials and methods

2.1. Micro organisms

Four strains of bacteria including *Lactobacillus acidophilus* (PTTC 1643), *Lactobacillus plantarum* (PTTC 1058), *Lactobacillus rhamnosus* (PTTC 1637) and *Lactobacillus lactis* (PTTC 1336), was prepared from Iranian Research Organization for Science and Technology (IROST) and they cultured according to the standard protocol. The bacteria were first inoculated in 50 ml of MRS medium and incubated for 48 hours at 37°C under anaerobic conditions and then solid culture was prepared in solid MRS medium for long term preservation.

2.2. Effect of drying on survival of probiotics

In this study, banana, apple, cucumber and apricot fruits were used. The fruits were washed with distilled water and disinfected with 70% ethanol and cut into pieces of 3.5 cm in diameter. Different Lactobacillus spp. in 50 ml of culture medium and 50 ml of MRS broth and 50 ml of Salt base culture medium (Table 1) and prepared and incubated in 24 hours at 37°C in anaerobic conditions. The bacterial growth was evaluated by spectrophotometry and pour plating method. Then, the cuts of the fruit were transferred to the two media containing the same bacterial contents to be Mixed for 5 minutes at 25°C (based on the Taguchi experimental design), then the fruits were transferred to a sterile plate and dried at different temperatures and drying periods (Table 1).

The drought process of the mixed fruits to bacteria was optimized based on the Taguchi experiment design methodology (Table 1) (Tille, 2015).

Dried fruit slices were transferred to liquid MRS broth and salt medium and completely immersed in the medium for 5 minutes at 25°C until all the bacteria adhered to the fruit surface. Pulp lining was done in sterile MRS medium and OD at 560 nm was measured for MRS broth and salt medium containing dried bacteria to determine the survival rate relative to the pre-dry conditions. Then, the MRS medium containing dried bacteria was incubated at 37°C for 48 hours to be used to determine the antimicrobial activity.

2.3. Salt culture medium Preparation

The salt base culture medium was used to study the growth and survival of probiotic bacteria under drought conditions at different temperatures (Kourkoutas et al., 2005).

2.4. Antimicrobial activity assay of Lactobacilli after drying

Antibacterial effect assay of Lactobacillus spp. before and after drying were determined. Four pathogenic bacterial strains including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 19659) were prepared. The antimicrobial activity of all Lactobacillus spp. were assayed by standard CLSI protocols.

2.5. Data analysis

The data were analyzed by Minitab software and the effect of bacterial species, time, drying temperature and type of dried fruits on the Lactobacillus survival and antimicrobial properties were evaluated. The significance level was considered to be less than 0.05.

Table 1. Taguchi experimental design for different essential factors

No	Time (h)	Temperature (°C)	Bacteria	fruits	OD in MRS culture medium	OD in salt culture medium
1	24	25	*L. acidophilus*	Cucumber	0.007	0.047
2	46	30	*L. rhamnosus*	Cucumber	1.563	0.811
3	68	35	*L. lactis*	Cucumber	0.653	0.871
4	90	40	*L. plantarum*	Cucumber	0.667	0.464
5	68	30	*L. acidophilus*	Apple	0.483	0.102
6	90	25	*L. rhamnosus*	Apple	0.189	0.085
7	24	40	*L. lactis*	Apple	0.990	0.984
8	46	35	*L. plantarum*	Apple	0.292	0.160
9	90	35	*L. acidophilus*	Banana	1.006	0.336
10	68	40	*L. rhamnosus*	Banana	1.658	1.406
11	46	25	*L. lactis*	Banana	0.392	0.321
12	24	30	*L. plantarum*	Banana	0.001	0.005
13	46	40	*L. acidophilus*	Apricot	0.620	1.013
14	24	35	*L. rhamnosus*	Apricot	1.591	0.537
15	90	30	*L. lactis*	Apricot	0.398	0.157
16	68	25	*L. plantarum*	Apricot	0.056	0.042

3. Results

3.1. Lactobacillus growth in MRS broth before and after drying

The highest and least growth of probiotics was observed in the presence of banana and apple respectively. The *Lactobacillus rhamnosus* and *Lactobacillus plantarum* showed the most and least growth. The best growth temperature for lactobacilli before drying was 40°C, and for 68 hours.

After drying, the highest and least growth of bacteria were observed in the presence of apple juice and apple fruit. The highest and least growth was related to *Lactobacillus plantarum* and *Lactobacillus acidophilus*, respectively showed the highest drying resistance. The best temperature and incubation time for

Lactobacillus plantarum were 25°C and 68 hours.

3.2. Growth in Salt base medium before and after drying

The results showed that the highest probiotic growth before drying were obtained in the presence of banana and the least growth was observed in the presence of apple. Before drying, *Lactobacillus rhamnosus* showed the highest growth and *Lactobacillus plantarum* had the least growth in salt medium. The best temperature for growth and temperature of Lactobacillus in salt medium were 40°C and 68 hours.

After drying, the highest and least survival was observed in the presence of apple juice and apple. *Lactobacillus rhamnosus* showed the highest growth after drying of fruits in salt medium and the least growth was attributed to *Lactobacillus acidophilus*. The best temperature and time for growth after the drying was 35°C and 68 hours.

MRS broth was a better environment for the growth of *Lactobacilli* with little difference before and after drying compared to the salt base medium. Data analysis also showed that fruits are suitable substrates for probiotic drying have survival potential in the culture media (Fig. 1-4).

Data analysis showed that in MRS and salt medium, the bacterial and temperature types had a significant relation with the survival of lactobacilli after drying, but the effect of different time and fruit type had no significant correlation with the survival of lactobacilli (Table 2).

In this study growth and survival of Lactobacilli in saline and MRS under dry conditions were studied. Since, based on the results, there was a very slight difference in the growth rate of lactobacilli before and after drying in saline and MRS, and the salt medium. Because of economical reasons the salt medium is more prefer than the MRS and it can be used for production Probiotic dried fruit on an industrial scale.

3.3. Evaluation of antimicrobial properties after drying

The results showed that lactobacilli had a significant antimicrobial effect against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* before and after drying. Before drying, *Lactobacillus plantarum* showed the highest antimicrobial activity against *Staphylococcus aureus*. *Pseudomonas aeruginosa* was the only strain sensitive to products produced by all lactobacilli. The details of the inhibition zone of growth before drying can be seen in Table 3.

The most antimicrobial properties after drying were obtained with *Lactobacillus rhamnosus*, at the apple against *Escherichia coli* at 20°C after 50 hours. Also, the highest resistance was found in *Bacillus subtilis*. On the other hand, *Pseudomonas aeruginosa* was more susceptible to lactobacillus products and drying in all 18 different conditions studied in this study. Details of the results of inhibition zone after drying under different conditions for each of the *Lactobacillus* species are present in Table 4.

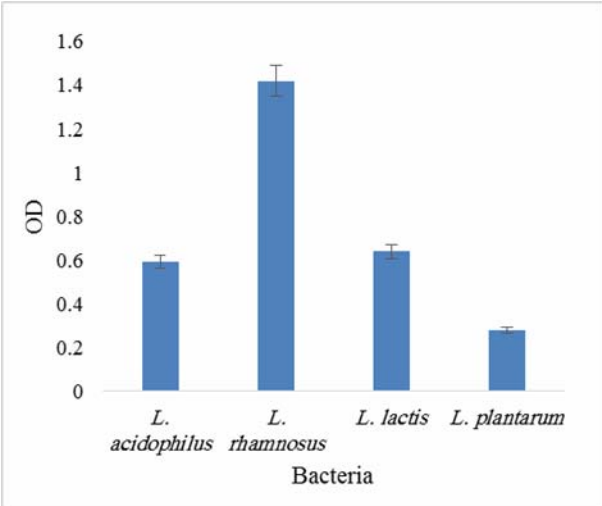

Fig 1. The relationship between bacterial type and growth rate (OD) in MRS culture medium.

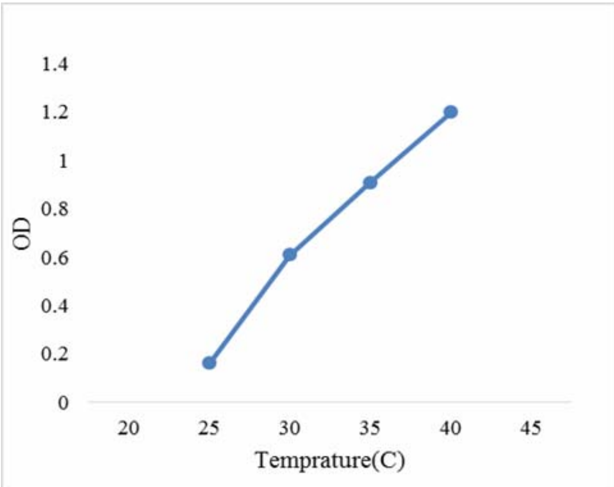

Fig 2. The relationship between fruits drying temperatures and growth rate (OD) in MRS culture medium.

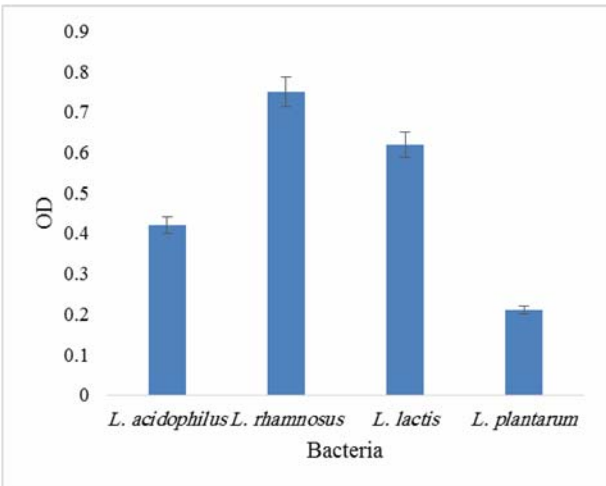

Fig 3. The relationship between bacterial type and growth rate (OD) in salt culture medium.

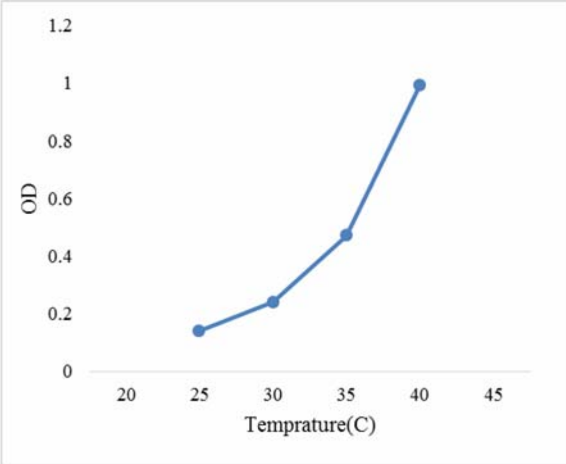

Fig 4. The relationship between fruits drying temperatures and growth rate (OD) in salt culture medium.

Table 2. Analysis of variance of data

Source	P-value (P <0.05) In MRS broth	P-value (P <0.05) In Salt medium	Seq SS	DF
Fruit type	0.849	0.386	0.17549	3
Bacterial type	0.032	0.042	2.13484	3
fruits drying temperatures (°C)	0.040	0.016	1.63591	3
Time(h)	0.960	0.137	0.06085	3

Table 3. Diameter of inhibition zone by different Lactobacillus spp. up on some pathogenic bacteria.

	Zone of inhibition (mm)			
Pathogenic bacteria / Lactobacillus spp.	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
L. acidophilus	9	8	0	0
L. rhamnosus	9.5	8	0	0
L. lactis	0	8	9.5	7.5
L. plantarum	0	7.5	10	9

Table 4. Details of inhibition zone in different situations by Lactobacillus spp.

N	Time(h)	Temperature(°C)	Bacteria	fruits	Zone of inhibition (mm)			
					E. coli	P. aeruginosa	S. aureus	B. subtilis
1	24	25	*L. acidophilus*	Cucumber	7.5	9	9	0
2	46	30	*L. rhamnosus*	Cucumber	0	8	0	0
3	68	35	*L. lactis*	Cucumber	7.5	8	0	8
4	90	40	*L. plantarum*	Cucumber	8	8	0	0
5	68	30	*L. acidophilus*	Apple	0	8	0	0
6	90	25	*L. rhamnosus*	Apple	10	7.5	8	0
7	24	40	*L. lactis*	Apple	8	9	0	0
8	46	35	*L. plantarum*	Apple	7.5	8	0	8
9	90	35	*L. acidophilus*	Banana	0	7.5	0	0
10	68	40	*L. rhamnosus*	Banana	8	8.5	0	0
11	46	25	*L. lactis*	Banana	7.5	9	9	0
12	24	30	*L. plantarum*	Banana	0	9	0	0
13	46	40	*L. acidophilus*	Apricot	8	8	0	0
14	24	35	*L. rhamnosus*	Apricot	0	7.5	0	8
15	90	30	*L. lactis*	Apricot	9	8	8	0
16	68	25	*L. plantarum*	Apricot	7.5	7.5	0	0

4. Discussion

Survival of probiotics is very important for their use in the treatment of diseases and the production of probiotic products. Increasing the probiotics survival by using different methods increases their function and ability, which can be used as an option for the prevention and treatment of pathogens (Meng et al., 2008; Ebel et al., 2014; Zayed & Roos, 2004; Huang et al., 2017).

Studies on the effect of drying and different variables on the survival and performance of probiotics are very low. Jaconelli et al. examined the effects of drying without using preservatives on probiotics and concluded that drying had an effect on the activity of bacteria and found a connection between different responses of probiotics and different methods of drying. (Iaconelli et al., 2015). In this study, due to the fact that the effect of drying on the antimicrobial activity of probiotic bacteria was evaluated qualitatively, the diameter of the inhibition zone created by dried probiotic bacteria decreased against pathogenic bacteria after than before drying and it can be concluded that drying has somewhat reduced its inhibitory effect. In other studies, Meng et al., using various methods such as spraying and freezing and dried probiotics concluded that drying is effective in the function of probiotic bacteria (Meng et al., 2008). In the present study, the comparison the amount of antimicrobial activity of lactobacilli before and after drying indicates the relative reduction of the antimicrobial activity of probiotic bacteria. Desmond et al., found that the probiotics survived during spray drying, and probiotics that were thermally compatible were more likely to survive (Desmond et al., 2001). In the present study, *Lactobacillus rhamnosus* showed the highest growth and survival in dry conditions compared with other tested lactobacilli.

Chávez et al., examined the probiotics survival in drying in different conditions and concluded that drying would preserve probiotics by freezing and spraying (Chavez & Ledeboer, 2007). Many studies have been carried out to assay the antimicrobial effects of Lactobacillus spp on pathogenic bacteria in Iran and other parts of the world. Shim et al., studied on the antimicrobial activity of Lactobacillus strains against urinary tract infection of *E. coli*, *Proteus vulgaris* and *Enterococcus faecalis* showed relative sensitivity against Lactobacillus products (Shim et al., 2016). In the study, the results of the inhibition zone of pathogenic bacteria before drying of probiotics showed that *Lactobacillus plantarum* and *lactococcus lactis* have the most antimicrobial properties against *Staphylococcus aureus*.

Naderi and colleagues studied the antimicrobial activity of probiotic lactobacilli on the urinary tract pathogenic bacteria with multiple resistance. Lactobacillus suspension has no antimicrobial activity against Enterococcus, Enterobacter and *Klebsiella pneumoniae*, but the antibacterial activity was significant against multiple drug resistant E. coli. (Naderi et al., 2014). In the present study, the results of the inhibition zone showed that the most antimicrobial activity after drying was related to *Lactobacillus rhamnosus* against E. coli. The results of the study by Forks et al. Showed that probiotics have antimicrobial activity against gastrointestinal pathogens, even those gastrointestinal pathogens that have multiple antibiotic resistance (Forks et al., 2017). Many researchers have shown the antimicrobial properties of Lactobacillus on nosocomial infection pathogens with high antibiotic resistance (Dallal et al., 2017; Lievin-Le, 2016; Likotrafiti et al., 2016). Ershadian et al. Also showed antimicrobial effects of Lactobacillus spp against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* (Ershadian et al., 2015). Based on the results of this research, lactobacilli showed significant antimicrobial properties against *Pseudomonas aeruginosa* before and after the drying process.

Conclusion

Data showed the MRS broth with a lower environmental difference is better for probiotic growth before and after drying than the salt medium. Also lactobacilli have antimicrobial properties against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* before and after drying.

Finally, the analysis of data revealed that dried fruits preserves the probiotics, before and after drying, the highest growth rate of bacteria was observed in 68 hours, indicating that they can be preserved by drying. After 68 hours incubation, they maximized their growth rate, after drying. Due to the fact that lactobacilli have significant antimicrobial effects against pathogenic bacteria, it is possible to preserve lactobacilli by using the drying method, and also to prepare probiotic dried fruit in the food industry.

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Refereces

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