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Original Research Article

INVESTIGATION OF PICKLED WATER SPINACH (*IPOMOEA AQUATIC)* FERMENTATION BY *LACTOBACILLUS* SP.

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Abstract. Extensive aquatic or semi-aquatic production of water spinach (*Ipomoea aquatica* Forssk.) for human consumption takes place in Vietnam.Pickled water spinach has rich iron content that makes it a good diertary food for people suffering from anemia. Its high level of S-methyl methionine is used in the treatment of gastric, intestinal problem, and hyperglycemia alleviation. We use the pure *Lactobacillus* sp. to ferment the water spinach pickle. Blanching is performed at 65-70^{o}C in 60 seconds with $CaCl_2$ 0.075% to improve product quality, especially structure and color. Fermentation batch is prepared with 3% salt, sugar 2%, lactic bacteria 0.75%. The water spinach pickle is preserved in potassium sorbate to extend its shelf-life.

Keywords:water spinach, blanching, lactobacillus sp., fermentation, shelf-life, pickle

1. Introduction

Water spinach (*Ipomoea aquatica*) is an aquatic plant found in marshy or wet sandy areas orfloating on water spinach (J. H. Bruemmer et al, 1979). Green leafy vegetables have been recognised as rich source of micronutrients (minerals and vitamins) and antioxidants (Kala and Prakash, 2004). Water spinach is a herbaceous perennial plant belonging to the family *Convolvulaceae*. It has a long, hollow and viny stem, grow prostrate or floating and the roots are produced from the nodes and penetrate into wet soil or mud.The leaves were found on dry weight basis to have high moisture, ash, crude lipid, crude fibre and available carbohydrate, but low in crude protein content. The leaves also have energy value. The mineral element contents were high with remarkable concentration of K and Fe. Also the leaves content moderate concentrations of Na, calcium,magnesium and P with low Cu, Mn and Zn contents (K.J. Umar et al., 2007).

The nutritional value of a particular food dependson its digestibility and its content of essential nutrients.Both digestibility and its nutrient content may beimproved by fermentation. During fermentation theenzymatic activity of microbial culture may

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predigestthe macronutrients (Kalantzopoulos, 1997). The different ways by which the fermentation process can affect the nutritional quality of foods include improving the nutrient density and increasing the amount of and bioavailability of nutrients. The latter may be achieved by degradation of anti-nutritional factors, pre-digestion of certain food components, synthesis of promoters for absorption and by influencing the uptake of nutrient by the mucosa (Svanberg, Lorri 1997).The lactic acid fermentation enhances protein solubility and the availability of limiting amino acids in somecases by as much as 50%. The micronutrient availability is also enhanced because of significant reductions inphytates. Tannins are reduced by as much as 50% and oligosaccharides by as much as 90% (Nout, Ngoddy 1997).

The nutritional impact of fermented foods on nutritional diseases can be direct or indirect. Food fermentationsthat increase the protein content or improve the balance of essential amino acids or their availability will have a direct curative effect. Similarly fermentations that increase the content or availability of vitamins such as thiamine, riboflavin, niacin or folic acid can have profound direct effects on the health of the consumers of such foods (Steinkraus, 1997).It was shown that lactic acid fermentation increased the utilisation of iron from food by breakaway of inorganicIron from complex substances under the influence of vitamin C (Siegenberg, 1991; Venkatesh, 1998).Fermentation may reduce the content of non-digestible material in plant foods such as cellulose, hemicelluloseand polygalacturonic and glucuronic acids. Breakdown of these compounds may lead to the improved bioavailabilityof mineral and trace elements (Kalantzopoulos 1997). Fermented foods may reduce the serum cholesterolconcentration by reducing the intestinal absorption of dietary and endogenous cholesterol or inhibiting cholesterolsynthesis in liver (Kalantzopoulos 1997). Lactic acid fermentation imparts attributes of robust stability and safety to the product, and thereby preempts disease infections such as diarrhoea and salmonellosis (Nout, Ngoddy 1997).

Purpose of our research is to investigate different factors affecting to the pickled water spinach fermentation such as blanching temperature and time; fermentation batch (lactic bacteria, salt, and sugar supplementation); preservation temperature and additive to its shelf-life.

2. Material & Method

2.1 Material

Green water spinach is purchased in the local market of Tra Vinh and Vinh Long province, Vietnam. Water spinach requires rapid and careful post-harvest handling to maintain quality. Once harvested, shoot tips and leaves easily wilt. So it's is necessary to cool in 50°F as soon as possible after harvest, and maintain high relative humidity with good ventilation. It should be conducted the experiments quickly after harvest to minimize quality loss

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Figure 1.Water spinach (*Ipomoea aquatica*)

2.2 Research method

2.2.1 Experiment #1: Effect of blanching temperature and time; $CaCl_2$content to product quality

Green fresh water spinach is cleaned with fresh water to remove foreign matter, dust and sand on its surface. Then it's sliced and size-formed before blanching and fermenting. The experiment is randomly arranged wuth 3 factors and two replication. Factor A: $CaCl_2$ supplementation (%) with three levels (A1: 0.05; A2: 0.075; A3: 0.1). Factor B: blanching temperature (^oC) with three levels (B1: 60-65^oC; B2: 65-70^oC; B3: 70-75^oC). Factor C: blanching time (second) with three levels (C1: 30 second; C2: 60 seconds; C3: 90 seconds). Total testings = A*B*C*n = 3*3*3*2 = 54. Testing parameters includecolor, structure, aroma and acidity in pickled product.

2.2.2 Experiement#2: Effect of lactic bacteria, sugar, salt to pickled product

Green fresh water spinach is treated, blanched as in optimal conditions in experiment #1. Then it's drained before fermentation. Experiment is randomly arranged with 3 factors and two replications. Factor E: lactic bacteria (%) with three levels (E1: 0.5%; E2: 0.75%; E3: 1.0%). Factor F: salt concentration (%) with three levels (F1: 2%; F2: 3%; F3: 4%). Factor G: sugar content (%) with three levels (G1: 1%; G2: 2%; G3: 3%). Total testings = = E*F*G*n = 3*3*3*2 = 54. Testing parameters include fermentation time (days), acidity in pickled product, and its sensory characteristics.

2.2.3 Product shelf-life by preservation temperature combined food additivie

Prepare the fermentation batch with conditions as in experiment#2. The fermented product is then rinsed with salt solution 2% and kept preservation. Experiment is randomly arranged with two factors, two replications. Factor M: Potassium sorbate (%) with 4 levels (M1: 0%; M2: 0.05%; M3: 0.075%; M4: 0.1%). Factor N: preservation temperature (^oC) with two levels (N1:5-7oC; N2: 28-32oC). Total testings= M*N*n = 4*2*2 = 16. Testing parameters include preservation time, and its sensory characteristics.

2.3Statistical analysis

All data are processed by ANOVA (Startgraphics) to check the significant difference via LSD.

3. Result & Discussion

3.1 Effect of thermal treatment to product quality

3.1.1Effect of $CaCl_2$supplementation into blanching solution to product quality

Table 1.Effect of $CaCl_2$ supplementation into blanching solution to product quality

$CaCl_2$ (%)	Firmness (g/mm^2)	Color (a index)	Color (b index)	Aroma	Acidity (%)
0.050	105^a	-3.89^a	20.70^a	3.31^a	0.87
0.075	109^a	-4.05^a	21.35^{ab}	3.56^b	0.85
0.100	110^a	-4.00^a	22.66^b	3.44^a	0.82
	F = 0.630	F = 0.55	F = 3.56	F = 3.09	F = 9.95
	P = 0.535	P = 0.581	P = 0.033	P = 0.047	P = 0.000

Water spinach after being fermented will become soft and less crispy. Using $CaCl_2$ can improve its crispness. Treated by 0.1% $CaCl_2$ product has better crispness than a sample treated by 0.075% and 0.05%. By fermenting with the same salt concentration, sugar and lactic bacteria; the lactic acid formation has changed towards decreasing when adding more $CaCl_2$. At 0.075% $CaCl_2$ product has a pleasant aroma. If we continue adding more CaCl2, product has astringent taste. However, the more CaCl2 we use, the more brightness of product we notice owing to Ca^{2+} separated.

3.1.2 Effect of blanching temperature to product quality

Table 2.Effect of blanching temperature to product quality

Blanching temperature (°C)	Firmness (g/mm²)	Color (a index)	Color (b index)	Aroma	Acidity (%)
60 - 65	118[a]	-3.82[a]	18.86[a]	3.20[a]	0.89[c]
65 - 70	115[a]	-4.08[a]	23.84[b]	3.66[bc]	0.84[b]
70 - 75	91[b]	-4.05[a]	22.01[b]	3.44[c]	0.81[a]
	F = 15.16	F = 1.2	F = 22.60	F = 10.35	F = 25.87
	P = 0.000	P = 0.306	P = 0.000	P = 0.000	P = 0.000

Blanching in high temperature will make product softer than blanching in low temperature. At 70 – 75°C blanching, the product has soft structure than product blanched at 60 – 65°C and 65 – 70°C. At 70 – 75°C blanching, product has better color than producted blanched at 60 – 65°C and 65-70°C. However in the fermentation, the trend will be decreased. Blanching at 65 – 70°C, lactic acid produces higher than 60-65°C and 70-75°C in fermentation. The reason of this phenomenon is due to high temperature probable of killing bacteria including *lactobacillus* sp. on raw material surface. When we add the same amount of salt, sugar and lactic bacteria, this can adapt to fermentation medium easily.

3.1.3Effect of blanching time to product quality

Table 3.Effect of blanching time to product quality

Blanching time (second)	Firmness (g/mm²)	Color (a index)	Color (b index)	Aroma	Acidity (%)
30	106[b]	-3.95[ab]	21.16[a]	3.32[a]	0.9[a]
60	125[a]	-4.27[a]	22.45[a]	3.67[b]	0.85[b]
90	93[c]	-3.72[b]	21.11[a]	3.32[a]	0.79[c]
	F = 19.09	F = 4.55	F = 2.05	F = 7.91	F = 49.61
	P = 0.000	P = 0.014	P = 0.136	P = 0.000	P = 0.000

Water spinach blanched in 90 seconds has bad structure owing to cell discruption and protopectin into soluble pectin. Water spinach blanched in 30 seconds has low firmness than in 60 seconds owing to not enough time for $CaCl_2$absorbed into material to interact with pectin to produce pectate calcium. Sample has the best firmness when being blanched in 60 seconds owing to the intact cell wall and CaCl2 penetrated and interacted with pectin inside tissue. The shorter the blanching (30 seconds) time is, the total acidity emits much more (90 seconds).

3.1.4 Effect of thermal treatment to firmness of water spinach

Table 4.Effect of thermal treatment to firmness of water spinach

$CaCl_2$	Blanching temperature (°C)	Blanching time (second)	Firmness (g/mm^2)
0.050	60 - 65	30	$112^{abcdefg}$
		60	116^{bcdefg}
		90	91^{abcd}
	65 - 70	30	$113^{abcdefg}$
		60	130^{defg}
		90	101^{abcdef}
	70 - 75	30	83^{a}
		60	104^{abcdef}
		90	81^{a}
0.075	60 - 65	30	123^{bcdefg}
		60	133^{defg}
		90	98^{abcde}
	65 - 70	30	$110^{abcdefg}$
		60	137^{defg}
		90	101^{abcdef}
	70 -75	30	87^{ab}
		60	105^{abcdef}
		90	81^{a}
0.100	60 - 65	30	128^{cdefg}
		60	139^{fg}
		90	$109^{abcdefg}$
	65 - 70	30	104^{abcdef}
		60	146^{g}
		90	90^{abc}
	70 - 75	30	91^{abcd}
		60	106^{abcdef}
		90	82^{a}
			F = 2.0
			P = 0.0397

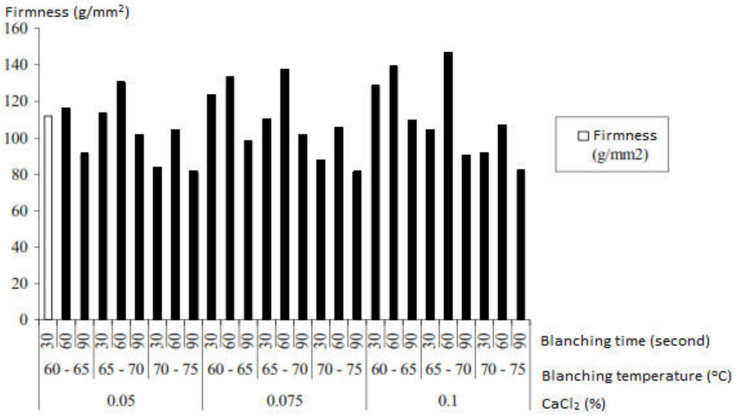


Figure 2. Firmness of blanched water spinach

The higher $CaCl_2$ content is, the more firmness of blanched water spinach is. Water spinach has the best firmness at $CaCl_2$0.1%. The higher temperature in blanching is, the less firmness of blanched water spinach is. Water spinach has the best firmness at 60 seconds.

3.1.5 Effect of thermal treatment to product aroma

Table 5. Aroma score of blanched water spinach

$CaCl_2$	Blanching temperature (°C)	Blanching time (second)	Aroma
0.050	60 - 65	30	2.75^{a}
		60	3.31^{abcdef}
		90	3.13^{abcd}
	65 - 70	30	3.38^{bcdef}
		60	3.81^{efg}
		90	3.38^{bcdef}
	70 - 75	30	3.31^{abcdef}
		60	3.44^{cdef}
		90	3.25^{abcde}
0.075	60 - 65	30	3.06^{abc}
		60	3.56^{cdef}
		90	3.44^{cdef}
	65 - 70	30	3.81^{efg}
		60	4.19^{g}
		90	3.38^{bcdef}
	70 - 75	30	3.50^{cdef}
		60	3.75^{efg}
		90	3.31^{abcdef}
0.100	60 - 65	30	2.81^{ab}
		60	3.44^{cdef}
		90	3.31^{abcdef}
	65 - 70	30	3.69^{defg}
		60	3.88^{fg}
		90	3.44^{cdef}
	70 - 75	30	3.56^{cdef}
		60	3.63^{cdefg}
		90	3.25^{abcde}
			F = 2.97
			P = 0.0018

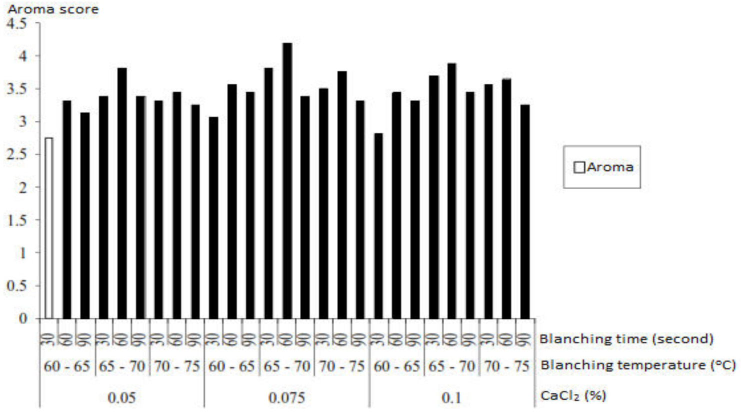


Figure 3. Aroma score of blanched water spinach

From figure 3, we can see the highest aroma score of blanched water spinach is defined at blanching temperature 65 - 70^{0}C in 60 seconds, $CaCl_2$0.075%.

3.1.6 Effect of thermal treatment to product acidity

Table 6. Acidity in the blanched water spinach

$CaCl_2$	Blanching temperature (^{o}C)	Blanching time (second)	Acidity (%)
0.050	60 - 65	30	0.98^{k}
		60	0.90^{fghijk}
		90	0.86^{cdefgh}
	65 - 70	30	0.93^{ijk}
		60	0.88^{efghij}
		90	0.80^{abcde}
	70 - 75	30	0.90^{ghijk}
		60	$0.84^{bcdefgh}$
		90	0.79^{abcd}
0.075	60 - 65	30	0.95^{jk}
		60	0.88^{efghij}
		90	$0.82^{abcdefg}$
	65 - 70	30	0.89^{fghijk}
		60	$0.85^{bcdefghi}$
		90	0.78^{abc}
	70 - 75	30	$0.87^{defghij}$
		60	$0.82^{abcdefg}$
		90	0.75 a
0.100	60 - 65	30	0.92^{hijk}
		60	0.88^{efghij}
		90	$0.83^{bcdefgh}$
	65 - 70	30	0.88^{efghij}
		60	0.81^{abcdef}
		90	0.77^{ab}
	70 - 75	30	$0.82^{abcdefg}$
		60	0.78^{abc}
		90	0.74^{a}
			F = 4.39
			P = 0.0001

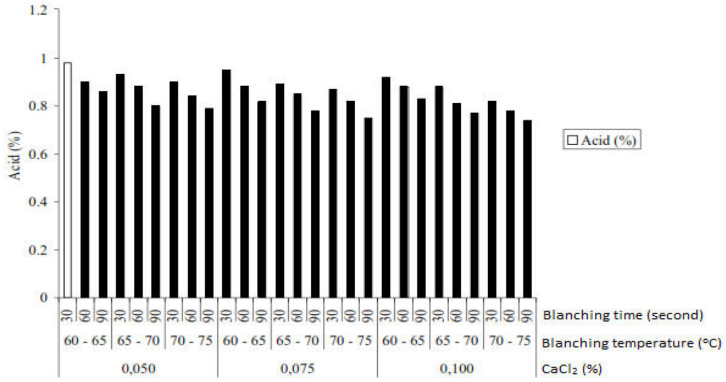


Figure 4. Acidity in blanched water spinach

With the same amount of salt, sugar and lactic bacteria, the sample blanched at temperature 60 - 65^{0}C, in 30 seconds has more lactic acid than in sample blanched at high temperature 70 – 75^{o}C in 90 seconds. The more $CaCl_2$ supplementation (0.1%) will limit lactic fermentation.

3.1.7 Effect of thermal treatment to color (a index) of blanched water spinach

Table 7. Color (a index) of blanched water spinach

$CaCl_2$	Blanching temperature (^{o}C)	Blanching time (second)	Color (a index)
0.050	60 - 65	30	- 4.32 abcde
		60	- 3.82 bcdefg
		90	- 3.80 cdefg
	65 - 70	30	- 3.16 fg
		60	- 4.96a
		90	- 3.52 efg
	70 - 75	30	- 4.02 bcdefg
		60	- 4.30 abcde
		90	- 3.13 g
0.075	60 - 65	30	- 4.32 abcde
		60	- 3.76 cdefg
		90	- 3.2 fg
	65 - 70	30	- 4.71 ab
		60	- 4.61 abc
		90	- 4.5 abc
	70 - 75	30	- 3.47 efg
		60	- 4.44 abcd
		90	- 3.7 cdefg
0.100	60 - 65	30	- 3.74 cdefg
		60	- 3.31 fg
		90	- 4.08 abcdef
	65 - 70	30	- 3.33 fg
		60	- 4.32 abcde
		90	- 3.54defg
	70 - 75	30	- 4.43 abcd
		60	- 4.93 a
		90	- 4.07 bcdefg
			F = 2.9
			P = 0.0005

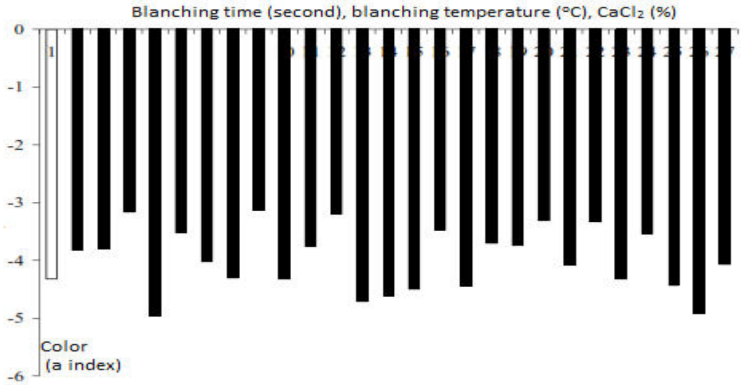


Figure 5. Color (a index) of blanched water spinach

3.1.8Effect of thermal treatment to color (b index) of blanched water spinach

Table 8.Effect of thermal treatment to color (b index) of blanched water spinach

$CaCl_2$	Blanching temperature (^oC)	Blanching time (second)	Color (b index)
0.050	60 - 65	30	16.99^{a}
		60	18.98^{abcd}
		90	18.63^{abcd}
	65 - 70	30	24.41^{efghi}
		60	22.23^{defgh}
		90	$21.48^{abcdefg}$
	70 -75	30	$21.20^{abcdefg}$
		60	$21.44^{abcdefg}$
		90	20.92^{abcdef}
0.075	60 - 65	30	17.03^{ab}
		60	18.70^{abcd}
		90	19.22^{abcd}
	65 - 70	30	24.00^{efghi}
		60	26.58^{hi}
		90	22.25^{defgh}
	70 - 75	30	$21.51^{abcdefg}$
		60	21.57^{bcdefg}
		90	$21.31^{abcdefg}$
0.100	60 - 65	30	17.58^{abc}
		60	19.95^{abcde}
		90	22.69^{defghi}
	65 - 70	30	24.76^{fghi}
		60	26.94^{i}
		90	21.90^{cdefg}
	70 - 75	30	22.95^{defghi}
		60	25.62^{ghi}
		90	$21.56^{abcdefg}$
			F = 2.79
			P = 0.0007

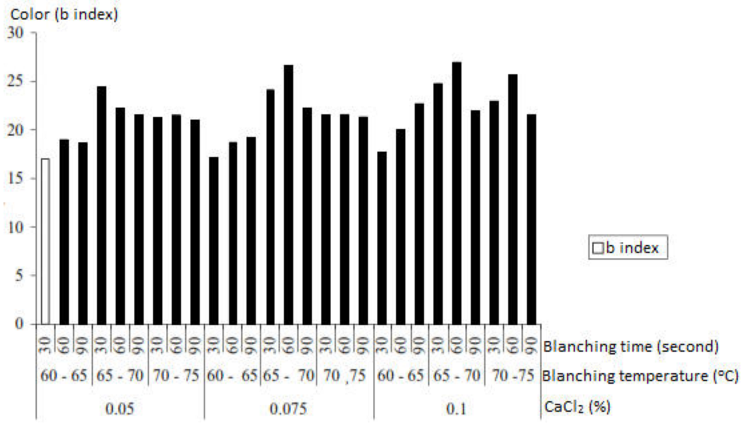


Figure 6. Color (b index) of blanched water spinach

Blanching at 65-70°C in 60 seconds, product has better color than blanching at 60 – 65°C and 70 – 75°C. In conclusion, blanching with $CaCl_2$ 0.075% in 60 seconds at 65 – 70°C is optimal.

3.2 Effect of fermentation condition to product quality

3.2.1 Effect of lactic bacteria to lactic acid formation during fermentation

Table 9.Effect of lactic bacteria to lactic acid formation during fermentation

Lactobacillus sp. (%)	Fermentation time (days)			
	1	2	3	4
	Acidity (%)			
0.50	0.26[a]	0.65[a]	0.73[a]	0.82[a]
0.75	0.28[a]	0.71[b]	0.79[b]	0.88[ab]
1.00	0.33[b]	0.79[c]	0.86[c]	0.93[b]
	F=28.50	F=24.26	F=15.01	F=9.90
	P=0.000	P=0.000	P=0.000	P=0.000

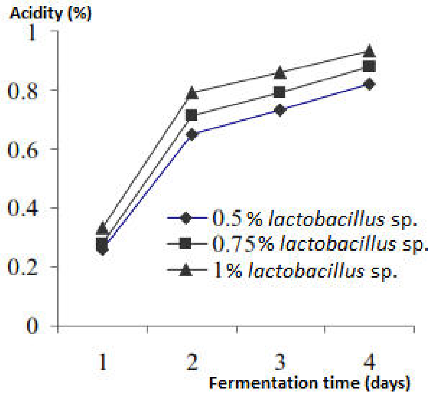


Figure 7. Effect of *lactobacillus* sp. to acidity formation during fermentation

We use more *lactobacillus* sp. we get more acidity in pickled water spinach. Moreover, sugar supplementation also enhances lactic fermentation. Acidity is highest at the 4th day of fermentation.

3.2.2 Effect of salt concentration to acidity during fermentation.

Table 10.Effect of salt concentration to acidity during fermentation

Salt concentration (%)	Fermentation time (days)			
	1	2	3	4
	Acidity in pickled water spinach (%)			
2	0.31[a]	0.75[a]	0.83[a]	0.93[a]
3	0.29[bc]	0.72[ab]	0.79[ab]	0.88[ab]
4	0.26[c]	0.69[b]	0.76[b]	0.83[b]
	F=13.23	F=5.20	F=4.28	F=6.72
	P=0.000	P=0.000	P=0.002	P=0.003

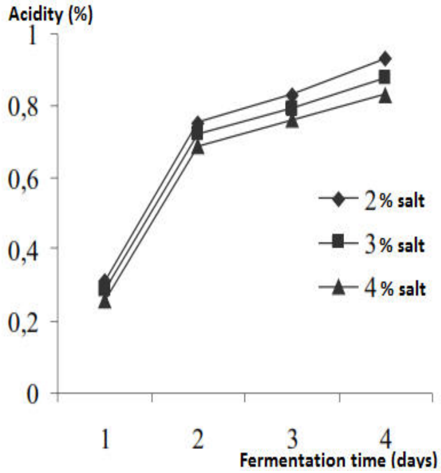


Figure 8. Effect of salt supplementation to acidity formation (%)

If we use more salt, lactic acid formation will be less owing to inhibition of high salt concentration.

3.2.3 Effect of sugar supplementation to acidity formation during fermentation

Table 11.Effect of sugar supplementation to acidity formation during fermentation

Sugar (%)	Fermentation time (days)			
	1	2	3	4
	Acidity (%)			
1	0.22[a]	0.65[a]	0.71[a]	0.79[a]
2	0.29[bc]	0.72[b]	0.80[b]	0.87[ab]
3	0.35[c]	0.78[b]	0.88[c]	0.97[b]
	F=98.54	F=20.62	F=24.32	F=24.48
	P=0.000	P=0.000	P=0.000	P=0.000

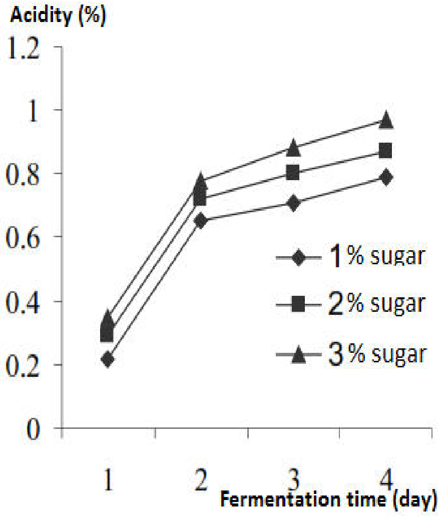


Figure 9. Effect of sugar supplementation to acidity (%) formation

If we use more sugar, we get more acidity %) owing to sugar as a substrate for*lactobacillus* sp. growth during fermentation. In conclusion, when we use more *lactobacillus* sp. and sugar we will get more lactic acid. The fermentation is optimal on the 4th day.

3.2.4 Lactobacillus sp. supplementation to product aroma

Table 12. Effect of *Lactobacillus* sp. supplementation to product aroma

***Lactobacillus* sp. (%)**	**Aroma**
0.50	3.4[a]
0.75	3.56[a]
1.00	3.29[b]
	F=3.72
	P=0.025

If we use more *Lactobacillus* sp. we will get more acidity. At 0.75% *Lactobacillus* sp. we get a pleasant sensory.

3.2.5 Effect of salt supplementation to product aroma

Table 13.Effect of salt supplementation to product aroma

Salt (%)	**Aroma**
2	3.23[a]
3	3.57[b]
4	3.44[ab]
	F=5.91
	P=0.000

When we use more salt, the acidity formation will be less. At 4% salt, there is no bad flavour owing to microbial inhibition but negatively affect to product sensory. At 2% salt, there is not enough to inhibit microorganism. At 3% salt, we get a pleasant feeling

3.2.6 Effect of sugar content to product aroma

Table 14.Effect of sugar content to product aroma

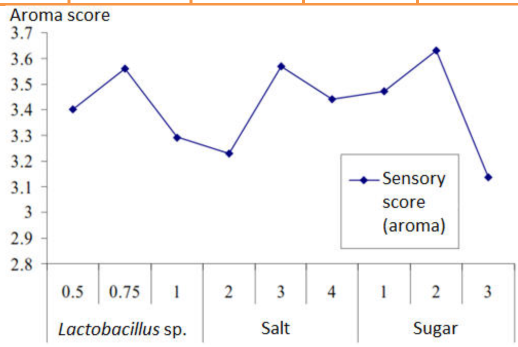
Sugar content (%)	Aroma
1	3.47ab
2	3.63a
3	3.14b
	F=12.63
	P=0.000

When we use more sugar, acidity formation will be increased. At 2% sugar, we get special aroma characteristics.

3.2.7 Effect of fermentation medium to product aroma

Table 15. Effect of fermentation medium to product aroma

Fermentation medium (%)	*Lactobacillus* sp. (%)			Salt (%)			Sugar (%)		
Culture (%)	0.5	0.75	1.0	2.0	3.0	4.0	1.0	2.0	3.0
Aroma	3.40[a]	3.56[a]	3.29[b]	3.23[a]	3.57[b]	3.44[ab]	3.47[ab]	3.63[a]	3.14[b]



Figre 10.Effect of fermentation medium to product aroma

3.3 Pickled water spinach shelf-life

Table 16.Pickled water spinach shelf-life

Potassium sorbate (%)	Preservation temperature (°C)	Shelf-life (week)
0	5 - 7	3
0	28 - 32	1
0.05	5 - 7	4
0.05	28 - 32	2
0.075	5 - 7	4
0.075	28 - 32	2
0.1	5 - 7	4
0.1	28 - 32	3

Preservation at low temperature combined with potassium sorbate can maintain aroma, color and structure of pickled water spinach. At potassium sorbate 0.05%, 0.075%, 0.1% combined low temperature 5 – 7°C after 4 weeks of preservation, the pickled water spinach still maintain color and aroma.

At normal temperature, shelf-life of pickled water spinach is downward after 3 weeks. At 28-32oC without potassium sorbate, we only preserve it in 1 week. At potassium sorbate 0.05% and 0.075% after 2 weeks the aroma, color and structure is downward. At 0.1% potassium sorbate after 3 weeks the pickled water spinach is still acceptable.

4. Conclusion

Fermented water spinach is food substrate that is invaded or overgrown by edible microorganisms whose enzymes,particularly amylases, proteases and lipases, hydrolyse polysaccharides, proteins and lipids to non-toxic productswith flavours, aromas and textures pleasant and attractive to the human consumer. The lactic acid fermentation of water spinach, applied as a preservation method for the production of finished and half-finished products, is considered as an important technology and it is further investigated because of the growing amount of raw materialsprocessed in this way in the food industry. The main reasons for this interest are the nutritional, physiological and hygienic aspects of the process and their corresponding implementation and production costs.

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