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Chemical and microbiological properties of pickling of caper (*Capparis ovata Desf. var. canescens*) fruits

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Abstract

The fruits of caper harvested in two different periods (July and August) were fermented in three different brines (5 %, 10 % and 15 %) for a month, and storaged for nine month. The chemical and microbiological properties of the brines were determined during both fermentation and storage. The highest pH was found in 3rd day of fermentation. In the beginning of the fermentation (1st day), acidity of brines for July period was higher than that of August ($p<0.05$). Acidity of all the brines gradually decreased in 30th day. In the end of the storage (3rd storage), the highest acidity was found in 5 % brine (about 0.70 %). Salt ratios of all the brines decreased in 3rd day of fermentation. Total bacteria (TB) number of brine increased in third and fourth day of fermentation compared to 1st day, and the highest level took in 21st day. The lowest and highest yeast-mould (YM) growth were in brines of 15 % and 5 % in the end of fermentation respectively. Lactic acid bacteria (LAB) growth wasn't seen in brines of 15 % during fermentation and storage. The highest LAB number was seen in brines of 5 % during fermentation.

Keywords: caper fruits, pickled, chemical and microbiological properties, and storage

1. Introduction

Caper has medical and aromatic properties [1,2]. Caper fruits are used for nutrition by preserving in brine or vinegar. In traditional method, young fruit is kept in water for 4-7 days, then the colour changes from green to yellow. In the second stage, the fruits are taken to brines of 10-12 %. After a while salt concentration is calibrated to 15 % (equilibrium) or a new brine (15-18 %) is prepared. Vinegar or spices such as tarragon may also be added to brine in consumer packs [3]. Caper pickling product is a quite expensive product and gives delicate flavours to very special food products. The sour, salty and astringent flavour is appetizing [4,5]. These values at pickled fruits of C.ovata were established as 69598 mg/Kg Na, 16761 mg/Kg K and 248.71 mg/Kg P, 30.36 mg/Kg Cu, 42.4 mg/Kg and 551.3 mg/Kg Zn [4]. Khurdiya and Verma [5] fermented the raw and mature fruits of *C. decidua* in brine for 1 month. After fermentation, the fruits are crushed, mixed with salt, spices, oil and vinegar, and kept at room temperature for 3 months. After storage, the sensory properties of samples are determined [5]. Characteristics of capers require more comprehensive studies for functioning and effective production. There isn't sufficient study about processing to pickled product of caper fruits. There is no commercial production of caper berries. There is little information on caper berries compared to caper buds. The aim of this study was to observe the chemical and microbiological properties of caper

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fruits (*Capparis* spp.) during fermentation and storage.

2.Material and Methods

2.1.Material

Unripe fruits of *C. ovata* Desf. var.*canescens* (Coss.) Heywood were harvested from Selçuklu (central county of Konya) in July and August 2006. The fruits were preserved in cooled condition until being taken to the laboratory. Clean spring water and rock salt were used for preparing the brine.

2.2.Method

2.2.1.Processing to pickled product of caper fruits and their storage

The fruits of *Capparis ovata Desf.*var. *canescens* were collected in several parties from the wild plants growing in Selçuklu, a central district of Konya. The fresh caper fruits were kept in cloth bags in refrigerator. While salt solution was being prepared, spring water and pickle salt without iodine. Caper fruits harvested in two different periods (July and August) were replaced into glass jars of 1 L, and they were completed by brines of 5 %, 10 % and 15 %. Caper fruits were processed to pickled product in their own harvest periods. All the samples were subjected to fermentation in incubator at 30 ^{O}C for a month. Brine analyzes (chemical and microbiological) were made once in two days in the first week and then once in a week for following the fermentation. Fermented fruits were preserved in their own brines at 30 ^{O}C for nine months, and chemical and microbiological analyzes of the brines were determined once in every three months in this period.

2.2.2. Chemical and microbiological analysis pH was measured by Basic Digital LCD-2 pH meter according to Cemeroğlu [6]. Acidity was determined as lactic acid (%) by Anonymous [7]. Salt analysis was determined according to AOAC [8]. In the microbiological analyzes applied to the brines, related microorganisms were determined by these media: Violet Red Bile Agar (Merck, Darmstadt) for coliform bacteria, Plate Count Agar (Merck, Darmstadt) for total bacteria, Potato Dextrose Agar (Merck, Darmstadt) for mould-yeast and Rogosa Agar (Merck, Darmstadt) for lactic acid bacteria. Microbiological analyzes were prepared from the dilutions of 10^{-2} with two repetitions. Incubation times for the microorganisms: coliform bacteria at 35^{O}C in anaerobic medium for two days, lactic acid bacteria at 30^{O}C in anaerobic medium for three days, total bacteria at 30^{O}C in aerobic medium for three days, mould-yeast at 22 ^{O}C in aerobic medium for five days. In the end of the incubation, colony numbers were determined by half-automatical colony counter, and expressed as cfu/mlx10^{2} [9,10].

2.3.Statistical analyzes

The study was regulated according to coincidence parcels 2 x 5 x 7 factorial test model. 2 was used as harvest period, 5 was used as type of brine and 7 was used as time. Results of the study were evaluated by variance analysis [11] and the differences between the groups were established by Duncan multiple comparison test [12].

3.Results and Discussion

3.1.Chemical properties of brines

Results of the chemical analyses applied to brine during fermentation and storage of caper fruits in the brines having different salt concentrations are given in Table 1. It was not found significant differences between pH values of 10 % and 15 % brines. The highest pH was found in 3rd day of fermentation. pH values of brines of July were found higher than those belong to brines of August. Small decrease and increase occured respectively in brines of July and August in the 5th day. pH values of the brines slightly slowed down in 2nd storage.

Among 5 %, 7% and 10 % brines, the most available fermentation was established in 5% [2]. While 10 % and 15 % brines are reaching the same pH value by a slight increase, pH of 5 % brine decreased. pH values of 5 % and 15 % brines belong to July period were found higher than those of August period. However, pH values of brines of 10 % belong to July and August are near the each other. pH of brines of August linearly increased proportional to salt concentration and then slightly increased. The lowest pH values were measured in 1st day belong to August brines of 5 % and 10 %. In the 3rd day, the lowest pH was seen in brine of 5 % belong to July period. pH values of all the brines showed parallel

increases to each other until 30th day. pH values of all the brines lowered in storage periods, the lowest pH was established in 5 % that belong to July in the end of storage. The pH was high in the 5% brine treatments during fermentation, and LAB grown at this concentration of brine during fermentation. LAB growth was not observed in 10% brine (except in the 10% brine, *C. ovata* treatment) after 10 days. High salt concentration obstructed activity of lactic acid bacteria, and prevented increase of acidity [13-16]. YM and Coliform bacteria (CB) did not grown after 10 and 15 days of fermentation, respectively [3,4]. According to variance analysis results, time and type of brine were effective ($p<0.05$) on acidity of brine, and effect of harvest period on acidity was found found significant.

In the beginning of the fermentation (1st day), acidity of brines of July period was higher than that of August. Brine of July showed a slight acidity decrease in 3rd day, and acidity value of this brine reached the highest level in 14th day by a gradual increase until the end of first two weeks of fermentation. In the beginning of fermentation, brines of 5% and 10% showed the same acidity (0.04 %), and the highest acidity was found in brine of 15 % (0.255 %). While acidity 10 % and 15 % brines decreased, acidity of 5 % brine increased in 21st day. The highest and lowest acidity values in the end of the fermentation were determined in 5 % and 15 % brines, respectively. Acidity of all the brines gradually decreased in 30th day, took the same value in 1st storage, but increased in 2nd storage. In the end of the storage (3rd storage), the highest acidity was found in 5 % brine (about 0.70 %). Acidity increased until 15 days of fermentation and was then stable. The highest acidity (0.80%) was determined for the 10% brine, *C. ovata* treatment after 15 days of fermentation [2].

Salt values of brines for July and August periods were found partly similar in 1st day. Salt values of these brines decreased in the same level in 3rd day, and had similar values in 5th day. Salt values of the brines belong to July and August periods showed slight changes in 7th day, 14th day, 21st day and 30th day. Salt values of all the brines decreased in 3rd storage. Salt values of 5 % and 10 % brines that belong to August were found respectively higher than those that of belong to July period. There isn't a considerable difference between salt values of 15 % brine for July and August periods. Salt ratios of brines of 15 % showed a rapid decrease in 3rd day, 5th day and 7th day. Salt ratios of brines of 15 % (July and August) showed parallel increases in 14th and 21st day. Salt values of these brines showed parallel decreases and took similar values in 3rd storage. Sanchez et al. [17] examined effects of brine buffering and different brine concentrations (0%, 4%, 7% and 10 % NaCl) on fermentation of caper fruit. They determined that brines of 7 % and 10 % prevented the fermentation and the samples with water and 4 % brine showed the same growth. The most suitable salt concentration in end product storage were reported as 10 %.

3.2.Microbiological properties of brines

Results of microbiological analyzes applied to brines caper fruits during fermentation and storage of caper fruits are given in Table 2. Total bacteria number of brine increased in third and fourth day of fermentation compared to 1st day, and the highest level took in 21st day. Increase of total bacteria number may result from decrease in salt ratio of brine in fermentation of 30 days. Differences between total bacteria numbers in first two weeks of fermentation and storage periods was not found statistically significant. From the variance analysis result, all of the factors were effective ($p<0.05$) on yeas-mould number of brine and interactions of Time x Harvest Period and Time x Type of Brine effected yeast-mould number.

Yeast-Mould growth slightly grown in brines of July and August in first three days of fermentation, this is thought to result from rapid pH decline in first three days. Yeast- Mould growth slightly occured in brines of August, and rapidly occured in brines of July period of storage. No yeast-mould growth is desired during fermentation. Because it may cause softening of product, color change, membrane formation in surface and then deterioration of product [18]. Mould-yeast numbers of 10 % and 15 % brines increased paralel to each other in 7th day and 14th day. The lowest and highest mould-yeast growth were in brines of 15% and 5% in the end of fermentation respectively. Brines of 10% and 15% are thought to be suitable with respect to product endurance and prevention of undesired formations.

All of the factors are effective ($p<0.05$) on lactic acid bacteria number of the brines. No lactic acid bacteria growth was seen in brines of July and August in 1st day, 3rd day and 5th day of fermentation. The highest lactic acid bacteria number was determined in 21st day and lactic acid bacteria number decreased in storage periods. Lactic acid bacteria numbers in August brines were found higher than those in July brines. This is thought to result from negative effect of pH changes on lactic acid bacteria growth and it's also thought that some other microorganisms cause pH lowering of brine in this period. A small increase of lactic acid bacteria number was in brines of July and a rapid increase happened in brines of August in 14th day and 21st day. This may probably result from pH increase in these days. Lactic acid bacteria numbers of brines of July and August lowered probably from acidity increase during fermentation.

Table 1. Chemical analyzes of brines during fermentation and storage of caper fruits

Time	Brine concentrations	Harvest Periods					
		July			August		
		pH	Acidity (%, lactic)	Salt (%)	pH	Acidity (%, lactic)	Salt (%)
1.day	5%	6.67±0.16*	0.04±0.00	5.00±0.00	5.39±0.03	0.04±0.004	5.00±0.00
	10%	6.72±005	0.04±0.04	10.00±0.00	5.51±0.03	0.02±0.00	10.00±0.00
	15%	6.98±0.02	0.5±0.00	15±0.00	5.83±0.06	0.01±0.001	15±0.00
3.day	5%	4.43±0.01	0.11±0.003	3.08±0.00	4.77±0.01	0.32±0.001	3.97±0.25
	10%	4.65±0.02	0.34±0.50	5.84±0.71	4.74±0.01	0.30±0.01	6.59±0.14
	15%	4.87±0.05	0.04±0.01	9.43±0.00	4.71±0.01	0.26 ±0.003	7.94±0.01
5.day	5%	4.54±0.03	0.39±0.03	2.94±0.00	4.90±0.03	0.33±0.01	3.58±0.10
	10%	4.58±0.01	0.28±0.04	4.58±0.00	4.83±0.01	0.31±0.02	6.37±0.00
	15%	4.57±0.02	0.23±0.04	8.34±0.00	4.8±0.02	0.3±0.001	7.38±0.10
7.day	5%	4.75±0.03	0.44±0.13	2.90±0.06	4.95±0.01	0.32±0.01	3.36±0.21
	10%	4.75±0.01	0.47±0.10	3.49±0.04	4.88±0.01	0.34±0.02	6.70±0.21
	15%	4.77±0.01	0.37±0.06	7.32±0.02	4.83±0.01	0.29±0.01	7.01±0.00
14.day	5%	4.86±0.04	0.73±0.08	2.76±0.04	5.12±0.02	0.66±0.01	3.65±0.21
	10%	4.92±0.01	0.86±0.21	3.52±0.08	5.12±0.01	0.63±0.004	6.21±0.10
	15%	4.94±0.01	0.77±0.20	7.21±0.06	4.92±0.08	0.63±0.01	7.6±0.00
21.day	5%	4.94±0.03	0.64±0.03	2.48±0.65	4.68±0.02	0.92±0.00	3.51±0.00
	10%	5.05±0.04	0.56±0.002	3.68±0.10	5.04±0.01	0.81±0.01	6.21±0.10
	15%	5.08±0.02	0.54±0.002	7.45±0.04	4.99±0	0.81±0.00	7.67±0.10
30.day	5%	4.83±0.01	0.61±0.02	2.93±0.02	4.15±0.51	0.81±0.40	3.5±0.60
	10%	5±0.01	0.55±0.007	3.74±0.02	4.80±0.01	0.39±0.01	6.14±0.00
	15%	5.02±0.04	0.44±0.007	7.34±0.00	5.05±0.03	0.31±0.02	7.16±0.21
1. Storage	5%	5.04±0.01	0.38±0.01	2.63±0.41	5.09±0.02	0.35±0.02	4.09±0.41
	10%	5.04±0.01	0.36±0.004	2.98±0.07	5.02±0.01	0.37±0.03	6.65±0.10
	15%	4.96±0.01	0.36±0.02	7.74±0.62	4.99±0.01	0.37±0.04	7.60±0.00
2. Storage	5%	4.94±0.00	0.41±0.01	2.92±0.00	4.30±0.01	0.54±0.01	4.02±0.10
	10%	4.93±0.00	0.41±0.011	3.21±0.00	4.88±0.02	0.34±0.01	6.65±0.10
	15%	4.88±0.00	0.45±0.03	7.74±0.21	4.87±0.01	0.39±0.02	7.6±0.00
3. Storage	5%	4.19±0.01	0.81±0.06	2.34±0.00	4.28±0.02	0.60±0.02	3.07±0.21
	10%	4.36±0.10	0.67±0.01	3.63±0.00	4.86±0.02	0.4±0.002	5.56±0.40
	15%	4.74±0.01	0.42±0.03	7.01±0.00	4.84±0.01	0.37±0.003	6.57±0.21

*mean±standard deviation

Table 2. Microbiologic analyzes of brines during fermentation and storage of caper fruits (- : non grown)

		Harvest Periods							
		July				August			
Time	Brine concentrations	Total Bacteria (cfu/mlx10^{2})	Yeast-Mold (cfu/mlx10^{2})	Lactic Acid Bacteria (cfu/mlx10^{2})	Coliform Bakteria (cfu/mlx10^{2})	Total Bacteria (cfu/mlx10^{2})	Yeast-Mold (cfu/mlx10^{2})	Lactic Acid Bacteria (cfu/mlx10^{2})	Coliform Bakteria (cfu/mlx10^{2})
1.day	5%	47.65±40.79	40.90±5.78	-	-	36.30±25.74	<10	-	<10
	10%	20.40±3.25	<10	-	-	24.95±22.56	<10	-	<10
	15%	22.65±6.44	13.55±6.43	-	-	109.05±10.92	-	-	<10
3. day	5%	768.15±96.38	<10	-	-	<10	-	-	-
	10%	309.05±3.34	67.32±1.10	-	-	-	-	-	<10
	15%	18.10±1.30	<10	-	-	<10	-	-	<10
5. day	5%	<10	-	-	-	201.25±1.77	2440±147.08	-	27.20±2.57
	10%	<10	<10	-	-	35.88±8.84	<10	-	13.6±0.00
	15%	<10	<10	-	-	474±33.94	81.75±64.28	-	29.50±3.53
7. day	5%	-	<10	-	-	125±35.36	1250±353.55	31.95±8.70	<10
	10%	<10	<10	-	-	190±14.14	272.5±32.17	-	43.15±9.69
	15%	<10	<10	-	-	336.25±22.98	210±49.50	-	65.85±54.66
14. day	5%	<10	<10	187.5±8.85	19.91±0.93	<10	477.25±372.86	350±197.99	-
	10%	<10	-	-	<10	2417.5±236.88	640.85±77.13	<10	-
	15%	<10	-	-	-	-	547.7±286.1	-	-
21. day	5%	498.46±4.33	447.70±13.81	425±77.78	<10	2018.75±235.98	50±7.10	15895.45±7064.63	-
	10%	670.4±41.50	747.70±42.75	-	18.15±6.43	4653.25±146.02	268.10±83.58	44.75±13.79	-
	15%	11429.5±161.20	122.7±32.10	-	<10	3490±14.14	195.4±25.74	-	-
30. day	5%	225±31.32	-	17.58±2.01	20.4±16.12	4000±1414.21	529.50±80.33	4143.15±1571.69	-
	10%	-	-	-	54.5±25.74	2250±353.55	302.25±31.17	2134.05±961.03	-
	15%	-	-	-	<10	1625±176.78	129.5±70.96	-	-
1.storage	5%	-	-	<10	-	103.04±15.23	14.0-±1.29	38.63±27.64	<10
	10%	-	-	-	-	<10	-	-	-
	15%	-	-	-	-	<10	-	-	-
2.storage	5%	1286.69±57.74	523.01±0.72	-	22.50±9.33	58.17±26.35	<10	-	15.67±4.18
	10%	2308.62±55.46	336.17±19.30	-	<10	107.95±41.46	<10	-	<10
	15%	543.4±12.63	630.09±23.80	-	<10	49.95±3.85	<10	-	<10
3.storage	5%	636.98±16.30	635.56±13.69	-	<10	<10	<10	-	<10
	10%	<10	-	-	<10	24.77±2.86	<10	-	<10
	15%	<10	<10	-	<10	41.57±25.50	<10	-	-

Lactic acid bacteria growth wasn't seen in brines of 15% during fermentation and storage. Growth was seen in brines of 5% from 7th day, reached the highest level in 21st day, lactic acid bacteria number decreased in 30th day and no growth was seen during storage periods. The highest lactic acid bacteria number was seen in brines of 5% during fermentation. The most suitable salt concentration for lactic acid bacteria growth until end of fermentation was 5 % [18]. It was reported that high salt concentrations in fermented products prevented lactic acid bacteria growth and low salt concentrations promoted the growth and provided high acid formation [19-21]. Özcan [2] reported that the most suitable salt concentration in fermentation of caper fruits with respect to lactic acid bacteria activity was 5-10 %. But fermentation in brine of 5 % must be taken under control because there's the risk of growth of unwanted microorganisms [18]. Increase of salt concentration stops activity of lactic acid bacteria and caused decrease of acidity. Furthermore, factors like enviromental conditions and low lactic acid bacteria number in microbial flora cause decrease of acidity [9,22-24].

It's seen that lactic acid bacteria growth in brines of 5% and 10% that belong to August was found quite higher than that of results in brines of 5 % and 10% that belong to July. This is thought to result from differences between acidity and salt values of brines and also from differences between lactic acid bacteria numbers in microfloras of caper fruits harvested in July and August. Alvarruiz et al. [14] found limited growth of various microbial species and only growth of some lactobacils and halotolerant cocci interesting inspite of sufficient reducing sugar content, pH value of 5.00 and salt concentration values of brine which is down to 5%. So it's thought that inhibitor compounds (such as glucocapparine) in caper fruits prevent microbial growth. The high salt concentration inhibited the growth of LAB [2]. Because the use of brine at a high concentration in pickling inhibits LAB growth, low concentration brines lead to microorganism growth and high acidity [17]. In studies examining 5%, the effects of 7.5 %, 10% and 20% brine, the most rapid fermentation occurred with a 5% salt concentration. According to other authors, LAB activity is not inhibited by high salt concentrations and increasing of brine acidity [2,14]. Sanchez et al. [17] found that in all cases of fermentation studied, LAB were the main

microorganisms responsible for the process. The results of the present study were generally similar to the literature findings, with minor differences due to different raw materials and processing parameters.

There isn't any coliform bacteria growth in brines of July in first week, and coliform bacteria number increased from 14th day to end of fermentation (also 30th day). Coliform bacteria (CB) growth in 7th day is thought to result from contamination during process. Little or no coliform bacteria growth is desired during fermentation. Coliform bacteria growth in a certain level is seen in 1st day in brines of August. The growth decreased in brines of August in 3rd day probably from pH decrease. Increasing of coliform bacteria number of these brines in 5th and 7th days can be probably result from pH increasing in this period. Coliform bacteria detected in brines may result from contaminants [18]. Özcan [2] reported that no growth of coliform bacteria was seen after 20th day of fermentation of caper fruits in brine of 10 %.

4. Conclusions

It is suitable to prefer brines of 5 % for August period with respect to acidity values. pH values of all the brines rapidly decreased in 3rd day of fermentation and slightly increased until 21st day (except slight decrease in brines of 5 %). Salt values of all the brines rapidly decreased in first three days of fermentation. No important changes happened in salt ratios of brines after 30rd day. Total bacteria growth of brines for July is more than that of brines of August in general. However, mould-yeast number of brines of August rapidly increased in 3rd day and mould-yeast growth of these brines is higher compared to brines of July until end of fermentation (except 21st day). Use of brines of 15 % and fruits of July is thought to be suitable with respect to microbial safety. Lactic acid bacteria growth was seen in brines of 5 % and 10 % after the first week of fermentation. The highest lactic acid bacteria growth was seen in briness of 5 % of August, growth is lower in brines of 10 % and increasing salt ratio negatively effected lactic acid bacteria growth. However, brines of 15 % of July are more safety with respect to growth of some unwanted microorganisms such as yeast-mould. No lactic acid bacteria growth was seen in brines of 15 % during fermentation and storage. In addition, more effective fermentation may ve provided by using starter cultures in brines of July to increase lactic acid bacteria growth.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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