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Examination of Nisin Effect on Time Reduction for Sterilization of Red Bean Conserve in Tomato Sauce

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Abstract: Although, the conservation process protects the foodstuffs from contaminations and increases the shelf life, however it causes unwanted changes in food materials. In this study, Nisin as a natural antivirus compound was used in order to reduce the effect of heat on physiochemical properties of red bean conserve in tomato sauce. To carry out this research, initially the exact duration of sterilization was determined in temperature at 121°C. Then Nisin in concentration of 2.5, 3.1, 3.7 and 4.3 ppm were added to samples. For each of these concentrations, 5 different durations for sterilization in 121°C were considered. Among these treatments, the samples which, like reference sample did not have any contamination and made continuation of other tests on them possible were only those and containing 3.1 and 3.7 ppm Nisin with 27.5 minutes sterilization, 2.5 and 3.1 ppm Nisin with 30 minutes sterilization and 2.5 ppm Nisin with 32.5 minutes sterilization duration respectively. The results showed that with lower levels of Nisin and higher levels sterilization duration contamination would decrease. Also various concentration of Nisin did not produce significant differences in levels of pH, salt and water drain weights of samples. Significant differences in colour factors (a* and L*) is related to sterilization duration which its increase is consistent with elevation in sterilization duration, the extent of light (L*), redness (a*) of samples. Nisin did not have any role in there changes. Accordingly contamination could be prevented with reduction in sterilization duration using 2 Hurdle, Nisin and heating.

Key words: Red bean conserve · Sterilization · Nisin · Hurdle technology

INTRODUCTION

Although heat process protect foodstuffs, increase the shelf life [1] it cost a lot of expenses in terms of energy, process leads to change in organoleptics and sensory properties of product in terms of flavour, colour and texture which in turn decrease consumers satisfaction. Also heat process destroys the nutritive materials of product such as protein, vitamin, carbohydrate and fat [2].

Currently demands for foods with least processing and without chemical materials are improving. To achieve this aim Hurdle technology could be employed which is a deliberate combination of the new existing conservation technology. The most important barriers and used Hurdles to conserve foodstuffs include: heat (low and high), water activity (a_w), pH, oxidation/reduction potential (Eh). In food processing, as high hydrostatic pressure, palsic electric field, radiation and natural biopreservative compounds could be used [3].

As examples of biopreservative compounds there produced by Lactic Acid bacteria or there biopreservative like bacteriocins could be mentioned which receive the most attention as biopreservative compounds with application in food, pharmacological and hygienic industry [4].

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Bacteriocins are made from Lactic Acid bacteria such as Lactococcus, Lactobacillus and Pediococcus. Nisin is one of the Bacteriocins which is a peptide with 34 groups A amino acid, Lantibiotics and produced by Lactococcus Lactis. Nisin has a bactericide effect on various positive gram bacteria such as Listeria monocytogenes and sporogenesis bacteria such as bacillus and clostridium species [4, 5].

In 1969 Food and Agriculture Organization (FAO) in America and World Health Organization (WHO) jointly confirmed usage of Nisin as natural biopreservative instead of chemical materials. Nisin posses useful features as a foodstuff biopreservative include being non toxic, natural, thermostable in low pH, very good storage capability, analytic capability by means of digestive enzymes and stability of taste and flavour in food. Nisin causes destruction of cytoplasmic membrane and leakage of useful materials such as ATP, amino acids and stream of small cytoplasmic compounds. Nisin perform this function by formation of pores in cytoplasmic membrane which in turn impede the biosynthesis process of the cell and destroy the bacteria [6].

The amount of applied Nisin in low acid conserves range 2.5 to 5 ppm. During the thermal process of low acid conserves, it is possible that some Nisin become eliminated. However, the truth regarding the effectiveness of low amount of Nisin, in fact, involves joint usage of Hurdles such as heat and a biopreservative agent such as Nisin. In other words, sensitivity of heat-damaged bacteria spores particularly for the positive gram thermophile bacteria are higher than that of Nisin [7].

In 1984, Morris and colleagues noted that the function of Nisin on spores occurs via connection to the groups of free sulfydryl protein. This issue shows that spores become more sensitive to Nisin when they are more damaged by heat; this is an important finding when Nisin is used as a biological preservative in heat processed food [8]. Also, in addition to protect foods from microbial contamination, the sensory and nutritive properties of the products are maintained and energy could be saved [9]. Researchers examined the effect of Nisin on various levels of heat and pH. They showed that in lower levels of pH, Nisin was more active than it was in higher levels of pH [8, 9].

Nisin has stronger antimicrobial effect on positive gram spores bacteria such as bacillus and clostridium than on the germination bacteria [10, 11].

This research aims to use Nisin as a natural biopreservative compound in order to reduce the duration of sterilization of red bean conserve. The study also is aimed to improve the sensory and organoleptics properties of the product, maintain its nutritive value and prevent over consumption of energy.

MATERIALS AND METHODS

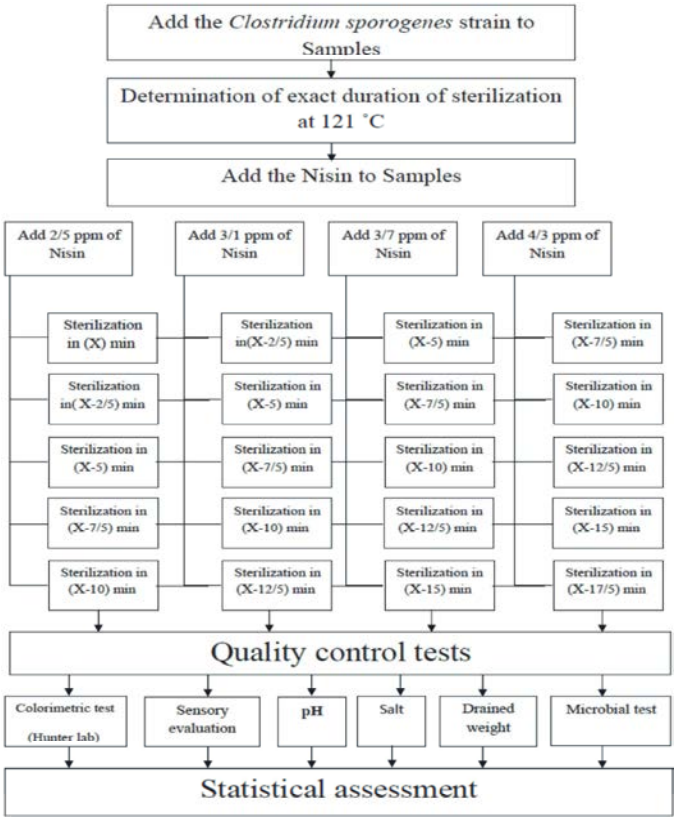
Materials: Nisaplin was obtained from Denmark Denisko Company and Nitric acid 65%, Aluminium ferric sulphate, Ammonium thiocyanate 0.1 N and Silver nitrate 0.1 N were obtained from Merck Company in Germany. Reinforced clostridial Agar (RCM), Peptone yeast extract bromocresol purple broth (PE-2) were obtained from Merck Company. Clostridium sporogenes bacteria PTCC 1651(*ATCC* 19404) produced by Iran microbial collection and all ingredients including red bean and tomato sauce were obtained from Behrooz food factory.

Methods

Clostridium Sporogenes Cultivation: Firstly Clostridium sporogenes has been prepared and transformed from the dry state through Ringer or physiology serum. Then obtained suspension is used to prepare single colony.

Determination of Exact Duration of Sterilization at 121°C: In order to determine the exact duration of sterilization for red bean conserve in tomato sauce, microbial suspension has been added to the samples, then after 30, 35, 40, 45 and 50 minutes sterilization at 121°C the samples have been cultivated. It was aimed to specify in which duration of sterilization any contamination would not exist.

Examination of the Nisin Effect on Time Reduction of Sterilization for Red Bean Conserve in Tomato Sauce: Initially, to prepare conserves within laboratory scales beans are blanched in steam with temperature of 100°C for 12 minutes, then the beans are poured in 410 gram cans and occupied thus 55% of their volume, tomato sauce filled the 45% rest of the can's volume. Then for each sample, 4 different concentration of Nisin including 2.5, 3.1, 3.7 and 4.3 ppm were considered. Each of the concentrations was sterilised in 5 different timing. It is necessary to mention that, given the logarithmic nature of destruction of bacterium due to heating, equal number of bacterium should be added to all conserve cans.



Microbila Test: To carry out microbial test, two samples were incubated at 30°C for 10 days and two samples were incubated at 55°C for 5-7 days. Also the samples were tested as to the existence of aerobic mesophilic bacteria, anaerobic mesophilic bacteria, aerobic thermophile bacteria and anaerobic thermophile bacteria.

The Physiochemical Test: The drained weight, pH, salt were measured.

The Colorimetric Test: Assessment of colour changes in produced samples was implemented. Also the effect of Nisin on the colour of product was examined through the function of Hunter Lab apparatus on tomato sauce.

Sensory Evaluation Test: In order to carry out the sensory evaluation of the selected samples, the Deming method was used. Namely for each sample, 5 factors including taste, colour, flavour, texture and viscosity were assessed.

Statistical Assessment: With respect to the distribution of data, two forms of statistical tests were performed. For the data with normal distribution a randomized design was employed in which treatments were combination of different duration of sterilization and various concentration of Nisin. If the distribution of data was not normal, the non-parametric test of Kruskal-Wallis was performed. To analyze the data SPSS and Minitab software were employed.

RESULTS AND DISCUSSION

Determination of Exact Duration of Sterilization at 121°C: To determine the exact duration of sterilization of red bean conserve in tomato sauce, microbial suspension with 10^4 CFU/ml microbial concentrations was inoculated to the cans containing conserve. At temperature of 121°C the cans were exposed to sterilization duration of shorter than 55 minutes. It was found that by reducing of time to 35 minutes, there was no bacterial growth. However, by decreasing of duration to 30 minutes, contamination was observed. In fact, with performing autoclave at temperature 121°C during 35 minutes, the sterilization of samples was completed. Thus, the result of the microbial test consistent with 2326 Iran standard was negative in that, there were no microbial growth. Accordingly, duration of 35 minutes was considered for sterilization of red bean conserve in tomato sauce (as the standard duration). Table 1 shows the results of microbial test of sample in order to find the exact duration of sterilization.

Table 1: The results of microbial test of samples in order to find the exact duration of sterilization at temperature 121°C

		Thermophiles		Mesophiles	
No	Samples	Anaerobic(*)	Aerobic (*)	Anaerobic(*)	Aerobic (*)
1	Control	N(*)	N(*)	N(*)	N(*)
2	Sample A with 50 minutes sterilization time	N(*)	N(*)	N(*)	N(*)
3	Sample B with 45 minutes sterilization time	N(*)	N(*)	N(*)	N(*)
4	Sample C with 40 minutes sterilization time	N(*)	N(*)	N(*)	N(*)
5	Sample D with 35 minutes sterilization time	N(*)	N(*)	N(*)	N(*)
6	Sample E with 30 minutes sterilization time	8	10	θ	θ

(*): Colonies per gram(cfu/gr) (*)N: No growth in the plate

θ: Swollen after 10 days Incubation

Note: All of the cans were prepared in the same condition

Table 2: The characteristic of produced treatment

No	Treatment	No	Treatment
1	Contaminated Control	12	22/5 min of Heating & 4/3 ppm of Nisin
2	Un Contaminated Control	13	25 min of Heating & 2/5 ppm of Nisin
3	15 min of Heating & 4/3 ppm of Nisin	14	25 min of Heating & 3/1 ppm of Nisin
4	17min of Heating & 3/7 ppm of Nisin	15	25 min of Heating & 3/7 ppm of Nisin
5	17 min of Heating & 4/3 ppm of Nisin	16	25 min of Heating & 4/3 ppm of Nisin
6	20 min of Heating & 3/1 ppm of Nisin	17	27/5 min of Heating & 2/5 ppm of Nisin
7	20 min of Heating & 3/7 ppm of Nisin	18	27/5 min of Heating & 3/1 ppm of Nisin
8	20 min of Heating & 4/3 ppm of Nisin	19	27/5 min of Heating & 3/7 ppm of Nisin
9	22/5 min of Heating & 2/5 ppm of Nisin	20	30 min of Heating & 2/5 ppm of Nisin
10	22/5 min of Heating & 3/1 ppm of Nisin	21	30 min of Heating & 3/1 ppm of Nisin
11	22/5 min of Heating & 3/7 ppm of Nisin	22	32/5 min of Heating & 2/5 ppm of Nisin

Table 3: The results of thermophile bacteria in produced treatments

	Thermophiles			Thermophiles	
No	Anaerobic(*)	Aerobic (*)	No	Anaerobic(*)	Aerobic (*)
1	1000	1000	12	15	13
2	N(*)	N(*)	13	13	12
3	420	389	14	4	4
4	190	155	15	2	2
5	95	93	16	1	1
6	61	59	17	N(*)	N(*)
7	45	43	18	N(*)	N(*)
8	43	38	19	N(*)	N(*)
9	32	30	20	N(*)	N(*)
10	21	21	21	N(*)	N(*)
11	17	15	22	N(*)	N(*)

(*): Colonies per gram(cfu/gr) N(*): No growth in the plate

Characteristics of Treatments: In order to show the various treatments within the results of this research, each treatment is coded in that, each treatment along with its characteristic is presented in Table 2.

The Results of Microbial Test: In this research in order to perform the microbial test, all treatments were incubated at 30°C and 55°C. Given that the optimum temperature of thermophile bacteria is 55°C, to examine their existence, they should be incubated at 55°C for 5-7 days. Table 3 shows that, although the amount of Nisin has increased, by decreasing the duration of sterilization at 121°C, the growth rate of bacteria has increased and the number of grown colonies in plates has risen. The reason is in the fact that the low acid such as red bean conserve in tomato sauce, due to obviation of sporogenes bacteria particularly thermophile bacteria which has more heat resistance than other bacteria, a high heating process is required [12], which by decreasing duration of sterilization this contamination has increased. As it is observed in

Table 4: The results of microbial test of mesophilic bacteria in produced treatments

	Mesophiles			Mesophiles	
No	Anaerobic(*)	Aerobic (*)	No	Anaerobic(*)	Aerobic (*)
1	1000	1000	12	θ	θ
2	N(*)	N(*)	13	θ	θ
3	θ	θ	14	θ	θ
4	θ	θ	15	θ	θ
5	θ	θ	16	θ	θ
6	θ	θ	17	N(*)	N(*)
7	θ	θ	18	N(*)	N(*)
8	θ	θ	19	N(*)	N(*)
9	θ	θ	20	N(*)	N(*)
10	θ	θ	21	N(*)	N(*)
11	θ	θ	22	N(*)	N(*)

(*): Colonies per gram(cfu/gr) N(*): No growth in the plate

θ: Swollen after 10 days Incubation

Table 3 and 4, by increasing duration of sterilization and decreasing in the added amount of Nisin, contamination will be eliminated and there will be no growth. During the heating process of low acid conserves, some amount of Nisin could be wasted but the fact is that a small amount of Nisin is still effective because bacterial spores particularly the positive gram thermophile bacteria, due to heating damage, become more sensitive to Nisin [7].

Mesophilic bacteria, because of their growth temperature at 37°C, will be incubated at 30°C for 10 days. According to Table 4, the results indicate that, at temperature 121°C, by decreasing duration sterilization from 35 minutes to 27.5 minutes and adding 3.1 ppm amount of Nisin, due to jointly usage of two Hurdles Nisin and heating, the growth of bacteria are stopped and not observed. However by decreasing the duration sterilization to 15 minutes swelling was observed in samples during the period of incubation. This swelling and inflate in could be due to degeneration accompanying the production of Carbon dioxide and Hydrogen in cans, or because by bacteria such as Sporogenes which has high level of heating resistance and is destroyed by sterilization in higher temperature.

As it is specified in tables, for some treatments, the results of microbial test are positive, or in other treatments swelling and inflation are observed. These treatments are omitted from the rest of the samples which had no contamination. Other tests were not performed on these treatments because on the basis of 2326 Iranian national standard, no growth should be observed inside the plates. Only these samples are usable which are free from any kind of thermophile and mesophilic bacteria. In addition to standard samples, 5 other samples has remained which, according to the standards, had no contamination and whose plates were devoid of colonies.

In fact, in this research both heat Hurdle and Nisin, as a natural biopreservative compound, were concurrently used with regard to what was observed when determining the exact timing of sterilization, by decreasing duration of sterilization to less than 35 minutes at temperature 121°C, aerobic thermophile bacteria, anaerobic thermophile bacteria, aerobic mesophilic bacteria and anaerobic mesophilic bacteria grew. However, the results showed that, by adding Nisin to 3.7 ppm to durations of less than 35 minutes no growth in bacteria was observed, of course this trend was maintained until the time reduction to 27.5 minutes at the presence of Nisin in amount of 3.1 ppm. However, when the sterilization time is decreased to less than 27.5 minutes, with any amount of used Nisin, contamination would appear. Accordingly, heat has an important role in destruction of microorganisms commensurate to various amount of Nisin.

For sterilization of low acid conserves such as red beans conserve in tomato sauce, the heating process equal to F0=6-8, in order to deactivate Clostridium botulinium spores and organisms producing degeneration are required. The work of other researchers indicate that, by adding of Nisin, the heating process could be decreased to F0=3 which in turn would improve the quality of low acid conservation products [13].

In general, consistent with previous researches, Nisin is effective in controlling the growth of positive gram spore bacteria such as Bacillus, Clostridium and also Listeria monocytogenes, Staphylococcus and many of lactic bacteria [14, 15, 9, 11].

During the heating process of low acid conserves, some amount of Nisin could be destroyed but the fact is that the small amount of Nisin is still effective, this stems from the fact that spores due to heating damage, become more sensitive to Nisin, particularly the sensitivity of positive gram thermophile bacteria [7, 16].

The Results of Physiochemical Tests: Physical and chemical tests on uncontaminated treatments according to Iran national 1635 standard were performed. Other swelled and contaminated cans were destroyed.

The Results of Measurement of Drained Weight: For each treatment, the drained water was measured for 5 times. The obtained results are shown in Table 5.

The results showed that, there is no significant difference between the treatments (P=0.05). Also various concentration of Nisin acted similarly during different duration of sterilization, the reason lies in the fact that the employed equal amount of Nisin are not the exact which that effect the samples drained water. The amount of drained water is influenced by the duration of soaking for bean and its blanching. The larger this duration, the bean become more swollen and it absorbs more water. Further, there is no significant difference among the weight of samples drained water. Thus this factor has not caused any difference in transmission of heat in conserve cans [17].

The Results of Acidity Test: According to the results of acidity test in Table 6, various treatments are not significantly different. In other words, the used amount of Nisin, as a natural biopreservative compound has no effect on pH because samples pH was not significantly different. Therefore, this factor which has a determining effect on thermo resistance of microorganisms has been similar in all tested conserve cans.

In 2003, in a research on pork which carried out by Baker and Nattress [18], pH differences were not due to Nisin but to the bacteria growth. This finding was confirmed by later studies [15, 11].

The Results of Salt Measurement: In this section, the amounts of salt in samples with various levels of Nisin and different duration of sterilization were compared. As it is observed in results of salt amounts in Table 7, the amounts of salt, as an influential fact for microorganisms activity is equal in all tested conserved cans. Elimination of microorganism has been affected by heating process and the amount of Nisin. Moreover, Nisin has no effect on foodstuff because it is natural and its composition is similar to foodstuff [6].

Table 5: The results of the Drained weight test

No. Treatment	Average of Drained weight
2	$61/4^{ns} \pm 1/14$
18	$61/6^{ns} \pm 1/14$
19	$60/6^{ns} \pm 0/89$
20	$60/8^{ns} \pm 0/83$
21	$61/2^{ns} \pm 1/30$
22	$61/4^{ns} \pm 1/67$

Data are expressed as average ± standard deviation.

ns: not significant at $P \geq 0.05$

Table 6: The results of pH measurement

No. Treatment	Average of Drained weight
2	$5/08^{ns} \pm 0/051$
18	$5/11^{ns} \pm 0/025$
19	$5/10^{ns} \pm 0/037$
20	$5/11^{ns} \pm 0/030$
21	$5/09^{ns} \pm 0/040$
22	$5/10^{ns} \pm 0/037$

Data are expressed as average ± standard deviation.

ns: not significant at $P \geq 0.05$

Table 7: The results of salt measurement

No. Treatment	Average of Drained weight
2	$1/21^{ns} \pm 0/008$
18	$1/21^{ns} \pm 0/016$
19	$1/21^{ns} \pm 0/016$
20	$1/21^{ns} \pm 0/021$
21	$1/20^{ns} \pm 0/021$
22	$1/21^{ns} \pm 0/028$

Data are expressed as average ± standard deviation.

ns: not significant at $P \geq 0.05$

Table 8: The results of Hunter Lab test

	Average of Hunter Lab		
No. Treatment	L^*	a^*	b^*
2	$26/52^{a} \pm 0/026$	$30/14^{f} \pm 0/046$	$30/28^{a} \pm 0/227$
22	$29/53^{b} \pm 0/161$	$28/89^{e} \pm 0/086$	$31/22^{b} \pm 0/040$
21	$29/89^{e} \pm 0/123$	$28/60^{d} \pm 0/113$	$33/21^{c} \pm 0/823$
20	$30/19^{d} \pm 0/047$	$27/76^{e} \pm 0/061$	$34/35^{d} \pm 0/282$
19	$30/70^{e} \pm 0/101$	$26/27^{b} \pm 0/287$	$34/15^{d} \pm 0/047$
18	$30/75^{e} \pm 0/041$	$25/21^{a} \pm 0/183$	$35/42^{e} \pm 0/153$

Data are expressed as average ± standard deviation.

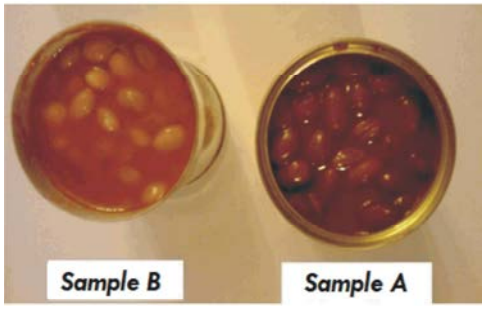
Values across the columns with different letters are significantly different ($P < 0.05$)

The Results of Colorimetric Test (Hunter Lab): In this section, the samples colour indices including L*, a* and b* were compared with standard sample. As it can be seen in Table 8, a* has been positive and shows the amount of redness and Lycopene remained in tomato sauce. In this respect there are significant differences between standard sample and other samples ($p<0.05$). The standard sample, with highest duration of sterilization, among other samples has had the highest level of a*. The amount of a* in other treatments has decreased. The samples 18 with 3.1 ppm Nicene and 27.5 minutes duration sterilization at temperature 121°C, has the least level of a*. This finding is due to the fact that by reducing duration of sterilization, the dark brown colour caused by long duration of sterilization would not be observed. It means, the lighter red coloured is observed in samples.

According to Table 8, L* has the least amount in standard sample. Except for treatment 18 with 3.1 ppm Nicene and 27.5 minutes duration which are equal on the basis of Kruskal-Wallis, there are no significant differences between standard sample and the rest of treatments. Among the treatments, the standard sample had the less lightness and treatments 19 and 18 had the most lightness. The extent to which the duration of sterilization is reduced the lightness becomes greater. As it is observed in Table 8, on the basis Kruskal-Wallis and in terms of b*, there are significant differences between treatments ($p<0.05$). Also, all treatments have various levels. The standard sample has the least amount of b* and sample 18 with the least duration sterilization of 27.5 minutes at temperature of 121°C and 3.1 ppm Nicene has the highest level of b*. The b* is related to the amount of Lycopene in very low extent. This case of unripe tomatoes or variety of tomato which has less Lycopene because it shows the yellow-blue colour [19]. In fact by increasing the level of L*, lightness of sauce which its dark redness (a*) is reduced, the amount of b* is rises.

The following picture shows two conserves, one of them has sterilized for 55 minutes (a) and the other one for 35 minutes (b). It can be noted that by decreasing the duration of sterilization of red bean conserve in tomato sauce there will be no basic colour change and darkening.

The differences between a* and L* does not stem from existence of different concentration of Nicene but it is related to the different duration of sterilization in the stable temperature of 121°C. As it has been mentioned earlier Lycopene causes redness in tomato sauce, which in natural state, it is in the form of isomer Trance, when



Picture 1: The sample (a) with 55 minutes duration of sterilization and sample (b) with 27.5 minutes duration of sterilization and 3.1 ppm Nisin

Lycopene is exposed to the high temperature of 121°C, its amount decreases. This reduction is due its transformation to Cis. This isomer is very unstable becomes oxidation in vicinity of Oxygen. As a result, in addition to the colour change of the product, its nutritive value also decreased [20].

The Results of Organoleptics Tests: To determination the consumer's favourite treatment, it is necessary to perform sensory assessment. On the basis of factors which in conserve factories in Iran have been determined the assessment standard of these products. The following properties are considered as the sensory assessment of treatments.

According to the results in Table 9, there were no significant differences between treatments in terms of colour, taste, flavour and appearance. The panellist's points did not differ significantly ($p\geq0.05$). The results showed that the different amount of Nicene have no effect on sensory properties of the samples. Also, the panellists commented that the obtained treatments, compared with samples acquired with long duration of sterilization had better colour and tomato sauce had preserved its red colour.

It appears that, due to reduction in duration of sterilization, the bad taste resulting from Maillard reaction did not occur. This in term resulted in higher points for smell and taste given that the amount of fat for red bean conserve in tomato sauce is low along with usage of Nicene, the duration of sterilization has reduced. Thus in the final product, there was no undesirable smell.

The treatments containing Nicene, for which the duration of sterilization was shorter, compared with similar sample excited in the market, had lesser mucilage and thus caused reduction of points for treatments. This issue

Table 9: The results of sensory assessment

	No. Treatment					
Parameters	2	18	19	20	21	22
Colour	$17/6^{ns} \pm 2/19$	$17/6^{ns} \pm 2/19$	$16/8^{ns} \pm 5/63$	$17/6^{ns} \pm 2/19$	$16^{ns} \pm 6/67$	$17/6^{ns} \pm 2/19$
Taste	$19^{ns} \pm 2/23$	$19^{ns} \pm 2/23$	$18^{ns} \pm 5/70$	$19^{ns} \pm 5/47$	$20^{ns} \pm 7/07$	$18^{ns} \pm 2$
Flavour	$19/4^{ns} \pm 4/66$	$19/4^{ns} \pm 4/66$	$15/8^{ns} \pm 2/86$	$19/4^{ns} \pm 4/66$	$17/4^{ns} \pm 2/40$	$19/4^{ns} \pm 4/66$
Appearance	$16/6^{ns} \pm 7/53$	$15/6^{ns} \pm 5/72$	$17/6^{ns} \pm 3/57$	$15/6^{ns} \pm 5/72$	$16/4^{ns} \pm 2/07$	$15/2^{ns} \pm 4/86$

Data are expressed as average ± standard deviation.

Values across the columns with different letters are significantly different (P <0.05)

resulted from the less cooking and lack of starches transfer to sauce phase. Here in several points should be made the acquired samples, compared with conserves with longer sterilization have the following privileges: being healthy, lack of crack on beans, no splitting and no accumulation of tomato sauce because of discharge of starch from inside of the bean due to long cooking and lack of pod without seed.

CONCLUSION

Taking into consideration the performed tests, it can be concluded that the exact duration of sterilization for red bean conserve in tomato sauce at 121°C was reduced pro 55 minutes to 35 minutes. At this time no contamination or microorganism growth occurred. The results of other performed tests were obtained according to the contents of 1635 Iranian national standard. Therefore, duration of 35 minutes was considered for sterilization.

Among the 22 produced samples only 6 treatments remained because, given the results of microbial test, by decreasing the duration of sterilization to 27.5 minutes at 121°C and increase in concentration of Nicene up to 3.1 ppm, there was no growth. In fact, it could be mentioned that the samples, without addition of Nicene, they will not have microbial growth for 35 minutes duration of sterilization, by adding the Nicene the duration could be decreased to 27.5 minutes. However, by adding more Nicene and decreasing the duration of sterilization to less than 27.5 minutes, microbial growth was occurred. Accordingly, combination of two Hurdle of heat and Nicene, the heat factor will be more effective than Nicene. Analysis of the results of physicochemical tests such as the drained weight, pH and salt content did not yielded any significant different between treatments, there finding indicates that Nicene has no effect on the drained weight, pH and salt content of foodstuff.

With respect to Hunter Lab test or colour assessment test in indices of a* and b*, significant differences were found between treatments. This issue stems from the fact that by decreasing duration of sterilization at stable temperature of 121°C, by preventing or decreasing the Maillard reaction, the darkness of the product lowers. Among the produced samples it is observed that by decreasing duration of sterilization, the amount of L* becomes greater than and darkness lowers and the level of a* decreases. In other words, the colour changes from dark red to light red, namely yellowness has increased. The sample 18 containing 3.1 ppm Nicene and 27.5 minutes duration of sterilization has highest and lowest level of L* and a* respectively. On the contrary, the standard sample with 35 minutes duration of sterilization have the least lightness L* and highest a*. It is necessary to mentioned that the existing differences in samples colour have no bearing on different concentrations of Nicene, colour is affected by different duration of sterilization. Although, samples on the basis of colour assessment through apparatus were different but on the basis of points for sensory assessment, they did not differ in terms of colour, taste, smell and appearance.

The reduction of duration of sterilization leads to decrease in intensity of Maillard reaction. Moreover, the volatile, smelly, bad taste compounds and also colour producing compounds such as Melanoidin do not appear. These factors have acquired high points.

Another aim of this research was to decrease the duration of sterilization of red bean conserves in tomato sauce by adding different concentration of Nicene in order to save energy. Taking in to account the results of this research it can be observed that relative to the duration of sterilization for standard sample which 35 minutes, 7.5 minutes reduction exists is. If in a factory with 16 autoclaves, each autoclave two times per day, totally 32 times autoclave is used. If these 32 times be multiplied by 7.5 minutes duration reduction, the duration of sterilization could be decreased 240 minutes per day. It means energy could be saved for 4 hours. This saving reduces the expenses and it is very cost effective for the factory.

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