ORIGINAL ARTICLE

Thermal Processing Determination Time for Fermented and Acidified Indigenes Iranian Vegetables

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Abstract

Vegetables may be preserved by fermentation, direct acidification, or a combination of these, along with pasteurization, to yield products with an extended shelf life and enhanced safety. Effect of lowering the pH, either by adding acid or lactic fermentation, on thermal process requirements for canned vegetables was investigated. Malic acid was preferred for acidification of canned vegetables. Addition of acid to covering brine was preferable to the blanching in acid solution, as the acidification was uniform, and it reduced the extent of discolouration. Acidification by lactic fermentation, using 2% hot brine to cover the prepared vegetable, reduced the pH to 3.8 in 3 days. Fermentation is initiated by the species of Leuconostoc and Lactobacillus followed by Pediococcus and Streptococcus, Process time, based on a sterilisation value of $F_{100}^{8.9}$= 3.5 min, was adequate to render the canned acidified vegetables (pH ≤ 4.0) microbiologically safe. The process time required for 77.8 x 119.1 mm and 103 .2 x 119.1 mm cans, having initial temperature of 65°C, was 15 min or less in boiling water. Colour of the lactic fermented canned products was superior to canned vegetables acidified with malic acid. Both had texture similar to that of the freshly cooked vegetables. Products acidified by fermentation had minimal sour taste.

Keywords: Acidified vegetables, Canning, Fermented vegetables, Microbial changes, Storage studies, Thermal processing.

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Introduction

Thermal process requirements for low-acid foods (PH > 4.6), especially vegetables , are severe due to the possibility of growth of toxigenic *Clostridium botulinum* and other heat resistant spore – forming bacteria FDA 2016. Acidification, either by addition of acid or by fermentation, to lower the PH as well as process requirements, modifies flavour or chelates trace metals (Saikia and Ranganna, 1992, Anon 1980, 1990; Kozup and Sistrunk 1982, Azizi, 2000).

Blanching in acid solution of PH> 2.0, immersing of blanched vegetable in acid solution or addition of acid to individual containers, are some of the direct acid addition methods for lowering the PH of the vegetables (Anon 1980). Citric, malic tartaric, lactic and acetic acids are generally used, while phosphoric, adipic, fumaric acids also find specific uses in the acidification of foods (Anon, 1990). Salad bar fresh process, developed recently, involves the addition of an ingredient having trade mark PHM-1 to the cans. This has been reported to reduce time as well as temperature of processing, and yield products, which are similar to freshly cooked vegetables, with respect to colour, texture and flavour (Anon, 1986).

Lactic acid fermentation also lowers the PH and, consequently renders the food resistant to pathogenic and spoilage microorganisms as well as inhibits the growth of spores (Anon 1980; Anderson et al. 1990; Cleveland et al. 2001; Suskovic, 2010). A number of vegetables are preserved by brining and fermentation. Cabbage and cucumber fermentation are, by far, the most extensively studied. Okra becomes soft and mushy on thermal processing and hence, is acidified, either by addition of acid or by lactic fermentation before canning. *Lactobacillus cellobiosus*, isolated from okra and when used as inoculums, reduces the pH to less than 4.5 after 24 h, and renders the surface of canned okra less mucilaginous and less sloughing (Kotzekido and Roukas 1987a). *L.plantarum*, *L.brevis* and *pediococcus cerevisiae* have also been used as start cultures in the fermentation of different vegetables (Fleming and McFeeters 1981; Breidt et al., 2013) and Toxigenic amines (1 to 5 mg/kg), formed during poisonous level (Anderson, 1988,).

In recent years, there is a renewed interest to reduce the thermal process requirements of low- acid (PH> 4.6) vegetables during canning, either by addition of acid (Kozup and Sistrunk 1982; Kotzekidou and Roukas 1987a) or by lactic fermentation (Kozup and Sistrunk 1982; Kotzekidou and Roukas 1987a, b; Edeza and Sanchez 1989; Anderson et al. 1990). Time and temperature conditions, which were made use of in thermal preservation by the authors, were found to vary considerably, and the basis for this has not been investigated. Studies on evolving thermal schedules for canned vegetables, acidified with acids or by fermentation, form the subject matter of this paper.

Materials and methods

Acidification using acids

Fresh vegetables, purchased from the local market, washed and used. The outer green leaves of cabbage removed, cored, and shredded. Carrots peeled and sliced to 1 cm thickness size. The stern of cauliflower was removed and the curds broken into pieces. The green beans were snipped and cut into small pieces of 5 cm length. Green peas shelled. Potatoes peeled, eyes trimmed and cut into halves. Kohlrabi were peeled and sliced to 1 cm thickness size

Prepared vegetables and brine (1%) blended in the ratio of 2:1 in a blender, and the blend acidified with 10% solution of the acid, to determine the quantity of acid required to get the desired pH.

For acidification, the prepared vegetables blanched in 3% solution of lactic, malic, succinic, citric and fumaric acids either for 15 min at 70 ^{0}C as recommended in the canning of cauliflower (Hoogzand and Doesburg 1961) or in boiling solution for 3 min, filled (500g) into A 2 $^{1}/_{2}$ (103.2 x 119.1 mm) plain cans, covered with 2 % salt solution, exhausted, sealed and processed in boiling water for 15 min.

Acidification by fermentation

The prepared green beans, cauliflower, peas, carrot and Kohlrabi in equal proportions or individually were filled (1.5 kg) into 5 litre jars and covered with hot (70 ^{0}C), coiled and cooled (45 ^{0}C), or cold (25 ^{0}C) brine of 2 to 6% concentrations, containing 0.4 to 0.8% acetic acid or without the acid. The containers were covered with nylon (200 Gauge) film and tied with thread. Use of hot brine with acetic acid is somewhat akin to the method flowed in Iran to preserve vegetables in the autumn for use in the winter.

Lactic acid bacteria were isolated from the fermenting vegetables at 12, 24 and 48 h intervals, using MRS broth and agar, (NCA, 1968), as well as *Lactobacillus- Streptococcus* differential medium (LSDM) (HiMedia, 1989). Isolation of pure cultures was done by serial dilution, plating and repeated streaking of single colonies. For biochemical studies, 12 h old cultures of individual isolates were purified by centrifugation. Identification procedure followed was according to Sharpe (1979) and Kandler and Weiss (1986).

After fermentation, the brine was drained,the fermented vegetable rinsed in water, filled (250 g) into 77.8 × 119.1 m or (500 g) into 103.2 × 119.1 mm cans, covered with boiling 1% brine, and the cans were exhausted, sealed and processed as before.

Thermal process evaluation

Heat penetration studies, using ecklund plug in needle type thermocouples and manually operated leeds and northrup potentiometer, indicated that the heating was by convection and the cold point was at about 1/10th the height of the can. Heat penetration data, collected at intervals of 1 min with 6 cans for each run, was used for calculating the process time required by equal time interval method (Patashnik, 1953) to inactivate the *Cl.pasteurianum* or heat resistant enzymes.

Inoculated pack studies

Cans filled with vegetables were inoculated with spores of *bacillus licheniformis* (7,50,000 spores/ can) and / or *cl.sporogenes* (2,40,000 spores/can). These cultures were isolated from canned mango pulp of pH 4.3 (Azizi and Ranganna 1993). The vegetables were then covered with brine, cans sealed and contents mixed. The inoculated cans were processed as described above and incubated at 37 ^{0}C uninoculated cans were used as control.

Storage study

The canned products were stored at room temperature and examined at intervals of 3 months. Texture was measured in Instron texturometer, using Kramer shear cell (2830-018) and plunger assembly (2830-010).

Results and discussion

Vegetables blanched in acid solution for 15 min at 70 ^{0}C, as recommended for cauliflower (Hoogzand and Doesburg 1961), or in boiling solution for 3 min, did not ensure acidification of the interior parts, but caused discolouration. The brown colour was nearer to the cut surface. Hence, acidification by addition of acid to the covering brine examined. Concentration of acid required to reduce the pH of the vegetables to 4.0 or less ranged from 0.06 to 0.55%, depending on the acid and the vegetable (Table 1). Sensory evaluation by ranking test (Ranganna, 1986) indicated that, among the acids studied, malic acid was the best acidulant for canned vegetables in brine, while malic acid made no difference for cooked vegetables.

The exploratory studies carried out using 2 to 6% brine, with added acetic acid (0.39 to 0.78%) or without acid at different temperatures, indicated that fermentation occurred, when 2% brine at 97 ^{0}C was used for covering the vegetables. The temperature had decreased to 55-58 ^{0}C on contact with vegetables within about 30 min. the use of hot brine probably destroyed some of the heat sensitive undesirable microorganisms and expelled cellular gases from vegetables. This favoured anaerobic fermentation, enhanced the stability of ascorbic acid as well as natural colour of the vegetables (Steinkraus, 1983), and inhibited the activity of enzymes. In the presence of hot brine, the waxy layer on the outer surface of the fresh vegetables was dissolved, thereby causing the natural colour to become bright. The fermentation rendered the vegetables translucent, texture remained firm and the vegetables as well as brine acquired the typical lactic fermented taste, but the brine was opaque in appearance. The fermented vegetables, produced using hot brine, were better than those involving the use of cold brine.

Vigorous fermentation was found to set in within 16 h of brining, the pH reduced at the end of 24 h to 4.1 – 4.2, and at the end of 90 h to 3.6-3.8. There was no change in the pH between 48 and 72 h. consequently, this period could be considered more as a period of

Table 1. Amount of Acid Required to Reduce the pH of Vegetable Brine Blend (2:1)

	Lactic acid		Malic acid		Succinic acid		Citric acid		Fumaric acid	
	%	pH	%	pH	%	pH	%	pH	%	pH
Carrot	0.15	3.8	0.10	3.9	0.22	3.7	0.13	3.8	0.28	4.1
Cauliflower	0.03	3.5	0.17	3.7	0.19	3.9	0.18	3.8	0.31	4.0
Green beans	0.22	4.0	0.12	3.8	0.27	3.9	0.01	3.7	0.32	3.7
Kohlrabi	0.19	3.5	0.06	3.8	0.12	3.7	0.07	3.7	0.22	3.8
Peas	0.28	4.1	0.25	3.8	0.40	3.7	0.27	3.9	0.29	4.0
potato	0.30	3.7	0.17	3.5	0.19	3.6	0.18	3.7	0.31	3.8

equilibration. After 24 h, the pH was lower, when boiling brine was used as compared to cold brine, but the brine had not penetrated into the interior of vegetables, particularly in those with a hard texture, such as carrot and cauliflower. Hence, fermentation for a minimum of 3 days was considered necessary.

Microbial changes during fermentation

The microbial flora of fermenting vegetables consisted of cocci and rods. The cocci in the first 12 h consisted of *Leuconostoc mesenteroides*. *L.cremoris* and *L.oenos,* which have the ability to withstand 60 c^0 for 30 min. these produced gas and acid, thereby contributing to anaerobic conditions, besides lowering the pH. Another species, the homofermentative *Lactobacillus delbrueckii* sub sp. Lactis, produced lactic acid during this period. Besides these microorganisms, during the first 24 h, the flora consisted of *pediococcus acidilactia*, *P.damnosus* and *Lactobacillus plantarum.* At the end of 48h, *sreptococcus lactis* and *S.rabbinolactis*, which produced only acid, dominated. The results show that, when hot brine was used, fermentation was initiated by *Leuconostoc*, which produced CO_2 and created anaerobic conditions. This organism, as well as acid producing lactobacilli, were followed by pediococci between 12 and 24 h. streptococci come into the picture only after this period. All these microorganisms contributed to the lowering of the pH to about 3.8 at the end of 3 days of fermentation.

pH of canned product

The vegetables fermented for 24, 48, 72 and 96 h, on rinsing and canning in fresh 1% brine. Had pH of 4.5, 4.3, 4.0 and 3.6 to 3.8, respectively. The pH of the vegetables, canned after 24h of fermentation, was close to 4.5, and even after 48h of fermentation, it was just 4.3. as these pH levels are favourable for the growth *B.coagulans* and *B.licheniformis* (Azizi and Ranganna, 1993) a fermentation period of 72 or 96 h is recommended. As it will lead to a pH of 4.0 or less in the canned products.

Thermal process requirements for canned products*:* National canners association (NCA, 1986) recommended sterilisation $F_{100}^{8.9}$ value equivalent to 0.1 min at pH 3.9 or less to 20 min at pH 4.4 – 4.5. $F_{100}^{8.9}$ of 20 min is equivalent to 3.5 min at 100°c. though the acidified vegetables had pH<4.0, $F_{100}^{8.9}$ of 3.5 min formed the basis for process evaluation in this study, to accommodate for variations in fill weight of vegetables, net weight, pH and also to impart cooked taste to the canned product. This F value is adequate to destroy *Cl.pasteurianum* (D_{100}= 0.1 to 0.5 min at pH 4.2 to 4.5) and non-spore forming yeasts and moulds (D_{65}= 0.5 to 1.0 min) (Stumbo 1973).

The graphical interpolation curves were given in Figs. 1 and 2. The time required in boiling water ranged from 12 to 15 min, except when the initial temperature was high (Table 2). Peas, french beans, Kohlrabi, cauliflower and carrot were found to support the growth of *B.licheniformis*, which elevates the pH, thereby resulting in conditions favourable for the growth of heat resistant *Clostidia* (Azizi and Ranganna, 1993). The D_{100} ranged from 2.1 to 2.8 in the vegetables investigated (Table 3). Process time to achieve sterilisation value, corresponding to 5D of *B.licheniformis*, would be considerably higher than the sterilisation value of $F_{100}^{8.9}$= 3.5 min. the redeeming feature is that the organism grows only at pH 4.2 and above. Hence, if the canned product is acidified to pH 4.0, with a safety margin of 0.2 units or even lower pH, the possibility of spoilage by this organism is forestalled.

Figs. 1 and 2. The graphical interpolation curves

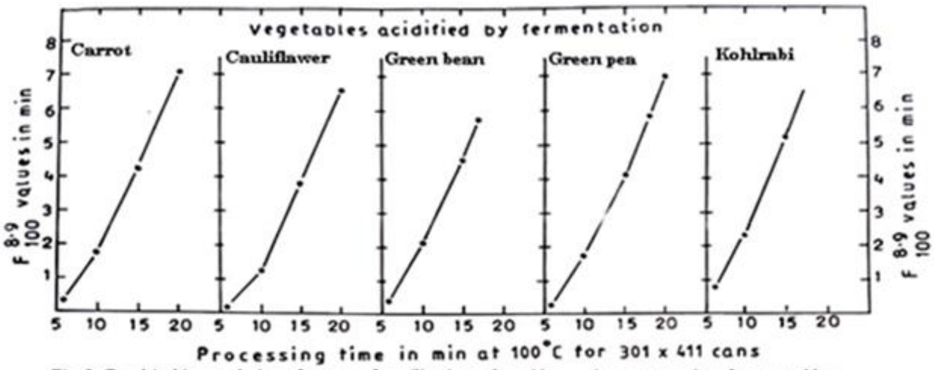

Fig. 1. Graphical interpolation of curves of sterilization values (i.e.) vs process time for vegetables acidified by fermentation and canned in brine in 77.8 x 119.1 mm cans.

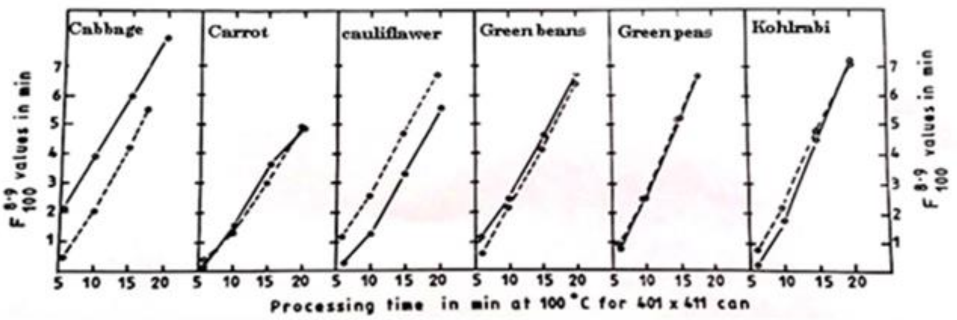

Fig. 2. Graphical interpolation curves of sterilization values vs processing time for fermented vegetables (O---O) and vegetables acidified with added acid (O----O) and canned in brine in 401 x 411 (103.2 x 119.1 mm) cans.

Table 2. Thermal Process Requirements at 97˚C For Canned Acidified Vegetables In Brine

Acidification	**pH**	**IT**	**77.8 x 119.1 mm cans processing time(min) corresponding to F of**		**IT**	**103.2 x 119.1 mm cans processing time (min) corresponding F of**	
		˚c	**3.5 min**	**5 min**	**˚c**	**3.5 min**	**5.0 min**
Acidufication using malic acid							
Cabbage	3.7		ND	ND	93	9	13
Carrot	3.9		ND	ND	64	15	18
Cauliflower	4.0		ND	ND	65	15	18
French beans	4.1		ND	ND	65	12	16
Peas	4.0		ND	ND	80	12	15
Kohlrabi	3.8		ND	ND	67	13	16
Acidification by fermentation							
Cabbage	3.6		ND	ND	63	13	15
Carrot	3.8	60	14.0	16.5	66	16	20
Cauliflower	3.8	64	15.0	17.5	66	13	16
French beans	3.8	65	12.5	16.0	66	14	17
Green peas	3.9	65	13.5	17.0	73	12	15
Kohlrabi	3.6	88	12.0	14.8	66	13	16

* IT:lnitial temperature at cold point of the can at the start of processing time

** ND: Not Determined. Acidity in the finished product ranged from 0.4 to 0.6%

Inoculated pack studies

No spoilage occurred in cans acidified to pH<4.0, inoculated with spores of *B.licheniformis* and/ or *Cl.sporogenes* and processed $F_{100}^{8.9}$=3.0 or 3.5 min, but this was not adequate for unacidified canned vegetables, and all such cans spoiled.

Changes during storage of the vegetables acidified using acid

When examined, soon after canning or after storage for 9 months, the vegetables were negative to heat resistant enzymes like catalase, preoxidase and polyphenoloxidase (Ranganna, 1986)

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Table 3. Thermal resistance (D) of B. Licheniformis in neutral phosphate buffer and vegetables

Product	PH	D_{100}VALUE (MIN)
Natural phosphate buffer	7.0	3.1 ± 0.20
Peas	4.2	2.5 ± 0.08
	4.5	2.5 ± 0.10
French beans	4.2	2.2 ± 0.20
	4.5	2.6 ± 0.20
Kohlrabi	4.2	2.2 ± 0.20
	4.5	2.3 ± 0.30
Cauliflower	4.2	2.1 ± 0.90
	4.5	2.8 ± 0.40
carrot	4.2	2.2 ± 0.20
	4.5	2.5 ± 0.20

Among the vegetables studied, the green colour of beans and peas turned yellowish brown during processing mainly due to chlorophyll destruction. This change was further intensified during storage. The natural creamy colour of acidified, canned cauliflower turned to light pink at the end of 3 months of storage and then to brown on further storage. The pink discolouration in cauliflower has been attributed to polymerisation of leucoanthocyanins present in a pseudo-base form (flavorn -4-ol), which is intensified further by the acid (Settee and Ranganna, 1972). However, carrot retained the natural colour over the prolonged storage. The texture of canned acidified vegetables was superior to canned unacidified vegetables processed under pressure, and resembled more closely to fresh cooked product, when examined at the end of 9 months storage (Table 4). Mere blanching of the fresh vegetables, a step in the conventional canning and in the canning of vegetables acidified using acid, considerably reduced the firmness, as seen from the minimum force required to begin extrusion (KN/kg) and the total work done (KJ/kg). Processing of the acidified vegetables in boiling water did not affect the texture further, whereas processing under 10 psig pressure (control) considerably affected the texture.

Acidified vegetables had perceptible sour taste. In the preparation of curried vegetables in Iran, acidulants like tomatoes, lime juice, dried lemone or unripe plum are added. Hence, slight sour taste should not alter the acceptability much.

Storage changes of canned vegetables acidified by fermentation

Irrespective of the colour of fresh vegetables, the fermented product, on canning appeared bright and retained the natural colour better. The change on colour during storage even up to 2 years was minimal. Since lactic acid was formed in situ from sugar by fermentation, the degradation of chlorophyll in green vegetables was minimal. Hence the colour of the canned product was dull green, but not brown, as in the case of vegetables acidified by acid. The values of texture properties of the canned fermented vegetables, after storage for 9 months, were lower than those of the fresh vegetable, but higher than those of blanched vegetable. This probably is due to exclusion of blanching in the canning of fermented vegetables (table 4). The overall quality of the canned fermented vegetable was far superior to the product canned using malic acid or by conventional method without acid, either immediately after canning or after prolonged storage. When made into curry, the lactic fermented note or sour taste was not distinguishable, as reported by the panel members to whom the canned fermented vegetables had been given for preparing the curried product, in their homes for evaluation purposes.

Conclusion

Vegetables acidified with malic acid, or by lactic fermentation, using 2% hot brine (the temperature decreasing to 55̊c on contact with prepared material) for 3-4 days, to lower the pH to 3.6-3.8, reduced the processing temperature by 15-20˚C and also the time. Similar work has been published by Kohajdova 2006. The resulting canned product is microbiologically safe, and similar in texture to fresh cooked vegetable.

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