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Assessment and Standardization of Microwave and Sodium Benzoate Treatments for Controlling Fermentation of Cauliflower Pickle

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Abstract

To control the over-acidification of fermented products has remained a challenging task for technologist and food processors. Standardization and optimal use of sodium benzoate and microwave treatment was carried out to control the fermentation of cauliflower pickle. The samples containing sodium benzoate at different concentrations S_1 (350 ppm), S_2 (450 ppm) and S_3 (550 ppm) and the samples treated with microwave heat M_1 (2.5 min), M_2 (3.5 min) and M_3 (4.5 min) for different time periods were compared with the control (C). The pH, titratable acidity (TA), texture and bacterial counts were periodically analyzed. The results showed that samples M_2 and M_3 showed significantly lower titratable acidity of 0.99 and 0.86, respectively and higher pH 4.76 and 4.33, respectively than the control (TA-1.45; pH- 3.70) throughout the 4 weeks of fermentation. Sample M_1 showed better results for pH (4.84), acidity (0.96), texture (6.6) and overall acceptability (8.1) than other treated samples. Samples S_1, S_2 and S_3 showed no significant difference to that of control. These results indicate that microwave treatment was able to arrest/slowdown fermentation of the pickle by inhibiting the growth of lactic acid bacteria (LABs), there by controlling the acid production.

Keywords: Fermentation, Microwave, Pickle, Sodium benzoate.

Due to diverse nutrient profile, vegetables are an important component of human diet. Not only that they are rich in phyto-chemicals and have been considered as important chemical reagents (Cordell *et al.* 2007) which improve human nutrition and health by various bioactivities. They represent a rich source of nutrients like carbohydrates, proteins, vitamins, minerals and fiber and non-nutrient phyto-chemicals such as sulfur containing chemicals that contribute to normal functioning of human body (Wettasinghe *et al.* 2002). These bioactive phyto-chemicals can modulate synthesis and absorption of cholesterol; reduce platelet aggregation and blood pressure. It has been reported that high consumption of vegetables significantly reduced the risk of cancer in humans (Block *et al.* 1992). Vegetables are also rich in antioxidants and consumption of these antioxidant-rich foods may improve antioxidant defense mechanism. Cauliflower (*Brassica oleraceae*) is one of the vegetables in the family *Brassicaceae*. It is low in fat and carbohydrates but high in dietary fiber, folate, water and vitamin C, possessing a high nutrient density. Cauliflower contains 4.97g carbohydrates, 1.9g sugars, 1.92g protein, 0.28g total

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fat, 0mg cholesterol, 2g dietary fiber and an energy value of 25 kcal. Fresh cauliflower is a rich source of vitamin C. It also contains good amount of B-complex vitamins like pantothenic acid (0.667mg), folate (57µg), pyridoxine (0.184mg), thiamine (0.050mg), niacin (0.507mg) and vitamin K (15.5µg) (USDA, 2009). Cauliflower contains several phytochemicals like kaempferol ($C_{15}H_{10}O_6$), quercetin ($C_{21}H_{20}O_{12}$) and a flavonoid isorhamnetol ($C_{16}H_{12}O_7$) which are common in the cabbage family that may be beneficial to human health (Vasanthi *et al.* 2009). Various studies regarding health benefits of cauliflower have demonstrated that they possess anti-proliferative activity (Dominique *et al.* 2009). Cauliflower heads contain several health benefiting phyto-nutrients such as indole-3-carbinol, 3, 3-di indolylmethane, sulphorane etc. that prevent overweight, diabetes and offer protection from prostrate cancers. Cauliflower contains in particular flavonoids and glucosinolates, which have been intensively researched for their health benefits (Hertog *et al.* 1995; Shivapriya *et al.* 2012). Cauliflower contains some enzymes which may interfere with thyroid hormone formation especially in people with iodine deficiency and hence can be goitrogenic (Rao 1995).

The vegetables once harvested are very much prone to spoilage due to their perishable nature. In order to make continuous supply of the nutritious and phyto-chemicals rich vegetables a need is felt to prevent them from immediate spoilage. Various processing methods have been applied to preserve and enhance the storage life of these vegetables. Fermentation has been considered as most viable processing method to make the vegetables preserved for consumption in off periods. Fermentation has been described as an energy yielding metabolism in which microbes incompletely oxidize the organic substrates which act as electron acceptors (Adams and Nout, 2001). Fermentation has been used as a method of preservation of perishable raw materials since Neolithic period (around 10000 years BC) (Prajapati and Nair, 2003). Fermentation inhibits the growth of pathogens and as such improves food safety (Adams and Mitchell, 2002). Fermentation not only preserves the food but also improves its nutrient content and their bioavailability due to the breakdown of anti-nutritional factors (Van Boekel *et al.* 2010; Sicard and Legras, 2011). Vegetables possess appreciable quantity of fermentable sugars, organic acids and amino acids for the growth and metabolism of LAB (Li, 2004) which significantly affects the flavour and sensory characteristics of vegetables. Since vegetables naturally harbor various LABs therefore as soon as they get the favorable conditions of salt concentration, anaerobic condition, water activity and temperature they undergo lactic acid fermentation spontaneously (Drosinos and Paramithiotis, 2007). Lactic acid fermentation of vegetables being traditionally a spontaneous and uncontrolled process results into undesirable and inconsistent products owing to the presence of diverse micro flora and various other variable factors. This has raised the necessity of adopting various strategies to control the lactic acid fermentation and the consequent product variability. The use of chemicals including sodium benzoate has been used widely to control the over-acidification of the fermented food product. However, the use of chemicals as such has not shown any significant results at the prescribed limits as evident from the literature. Microwave may be used to control the over-acidification of the food product by either slowdown or arrest the growth of microbes.

The main aim of the current study was therefore to control or slowdown fermentation of vegetables (cauliflower) after achieving a desired level of acidity so as to enhance the shelf life of the product and maintain its consumer acceptability during storage and marketing.

MATERIALS AND METHODS

(a) Raw material

Cauliflower was procured from local market and was brought to the Food processing pilot plant of the department of Food science and Technology. The vegetables which were free from physical damage, dirt, dust and other defects were used for the product formulation.

(b) Chemicals and reagents

The chemicals and reagents used for Physico-chemical and microbial were purchased from Sigma-Aldrich Chemie (Buchs, Switzerland), Himedia India. All the chemicals were of analytical grade.

(c) Product formulation

The vegetables were thoroughly washed under tap water. Surface leaves of cauliflower were removed and florets were separated from the central stalk. The cauliflowers were peeled and tops were cut off. Washed cauliflowers were shredded into pieces (3-4 cm in length and 1 cm thick) and were weighed. The spices like ginger and garlic were peeled, chopped and then coarsely pounded. Mustard, red chilli and turmeric were used in powder form.

Table 1: Composition and quantity of spices used in the preparation of cauliflower pickle

Sl. No.	Spice	Quantity (g/1000g)
01	Red chilli powder	12
02	Turmeric powder	10
03	Mustard seeds	35
04	Carom seeds	35
05	Garlic	30
06	Ginger	30
07	Mustard oil	140

er, carom seeds were used in intact form. Shredded vegetables were dried under sunlight for 3 hours in order to remove the surface moisture with periodic turnings. The dried vegetables were mixed with specific quantity of spices and other ingredients (Table 1). 3% salt was added to the mixture on w/w basis. The product so obtained was then filled in previously sterilized plastic containers. The filled containers were stored in the incubator (Memmert INE 500, Germany) at 21 ^{0}C. The containers were monitored continuously until desirable acidity was achieved. The fermented products were divided into two portions. One portion of them was subjected to sodium benzoate treatment at concentrations of 350, 450 and 550 ppm while as the other was subjected to microwave treatment for 2.5, 3.5 and 4.5 min. The treated products were then filled into glass bottles separately and was labeled as S_1 (350 ppm), S_2 (450 ppm) S_3 (550 ppm) M_1 (2.5 min), M_2 (3.5 min) and M_3 (4.5 min), respectively. One bottle kept untreated was used as control (C).

(d) pH analysis

The pH measurements of samples were carried out at regular intervals of time. Samples were homogenized (wise TIS homogenizer HG-15A, Korea) and left undisturbed for 20 minutes. The samples were centrifuged at 1200 rpm for 20 minutes. The supernatant obtained was used for the analysis of pH. The pH of the samples was determined by pH meter (HANNA-HI 2215 pH/ORP meter, Romania).

(e) Titratable acidity (TA)

Titratable acidity of the samples was determined by titrimetric method. Sample of 10 gm was homogenized (Wise TIS homogenizer HG-15A, Korea) with 100 ml of distilled water. To the 10 ml of aliquot, few drops (2-3) of phenolphthalein was added as an indicator. The acids (lactic acid) in the samples were titrated against 0.1N NaOH and expressed as mg lactic acid 100 gm of fermented pickle extracts. The percentage of the lactic acid was calculated as follows:

$$\text{Acidity \%} = \frac{\text{N} \times \text{Eq.Wt. of acid} \times \text{Vol. made}}{\text{Wt. of sample} \times \text{Vol. of aliquot} \times 1000} \times 100$$

(f) Microbiological analysis

Total plate count of each sample was done aseptically by transferring 1 mL of sample into 9 mL of sterile Peptone water buffered (Biolab, Merck, Germany). The contents were mixed thoroughly. MRS agar was used as nutrient media for the enumeration. Serial dilutions for each of the sample were done according to. All the glassware used during the microbial analysis was pre-sterilized. Plates were incubated at 37 ^{0}C for 48 hours. The microbial load was expressed as $\log_{10}$ cfu/g for each sample.

(g) Instrumental color

The color values of the fermented pickle samples during the storage period were determined using a Hunter Color Lab (Mini Scan XE Plus, model No. 45/0-L, Hunter Associates Laboratory, Reston,VA). The instrument was calibrated with black and white tiles before color measurement. The "*L**" value indicates the lightness, with 0–100 representing dark to light. The "*a**" value gives the degree of the red–green color, with a higher positive "*a*" value indicating more red. The "*b**" value indicates the degree of the yellow–blue colour, with a higher positive "*b*" value indicating more yellow. The average value of three replicates was calculated.

(h) Texture analysis

The textural analysis of the each sample was done by 5-blade Kramer share cell (HDP/KS5) using texture analyzer (Stable micro system, Model TA.XT plus, England). Texture profile analysis (TPA) was performed in duplicate on each sample at room temperature. Samples were taken from the center of each glass bottle. The conditions for texture analysis were as follows: test speed 3.00 mm/sec, post- test speed 10.00 mm/sec, maximum load 50 kg, distance 25 mm and trigger type 'button'. Prior to analysis, the height of the blades was calibrated. The texture profile analysis was obtained by graphing a curve using force (g) and time (sec) plots. A value of mean maximum force (firmness) was determined for each sample.

(i) Sensory analysis

The sensory analysis of the fermented product was done on the basis of 9- point hedonic scale for attributes like, flavour, sourness, firmness and color. Sensory analyses were carried out by the research scholars and faculty members (25 to 48 years of age) in the Department of Food Science and Technology. Samples were coded and served at room temperature with water. Samples were given to panelists separately for unbiased evaluation of sensory attributes. Taste was evaluated as sourness, texture as firmness, flavour intensity. However, overall acceptability was evaluated as the sum of all the sensory attributes considered and was calculated.

(j) Statistical analyses

The statistical analyses were performed using the IBM SPSS statistics 21. The differences among the treatments were evaluated statistically by one-way analysis of variance (ANOVA) and Duncan's multiple tests. All data were two sided at the 5% significance level and are reported as means ± standard deviations (SDs).

RESULTS AND DISCUSSION

(a) Microbial stability

The values of the microbial count for the different samples are given in the Fig. 1.

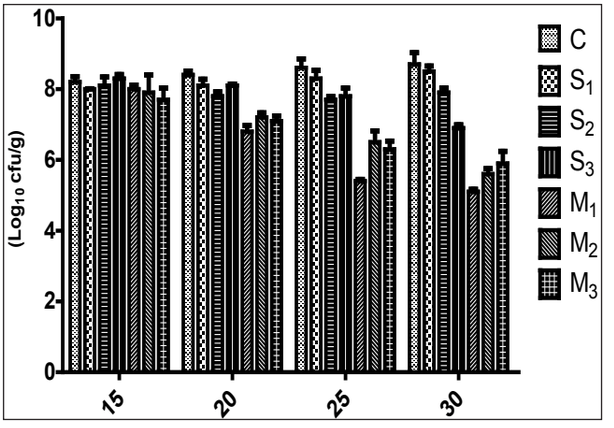

Fig. 1: Microbial load of different samples of cauliflower pickle

The microbial count in the control (C) was found to increase rapidly reaching to a value of 8.2 $\log_{10}$ cfu/g at the 15-day of fermentation and continued to increase to 8.7 $\log_{10}$ cfu/g at 30-day of fermentation. Microbial count increased non-significantly in S_1 (8.0-8.5 $\log_{10}$ cfu/g), S_2 (8.1-7.7 $\log_{10}$ cfu/g) and S_3 (8.3-6.9 $\log_{10}$ cfu/g) as compared to control indicating that sodium benzoate up to concentration of 550 ppm does not show any inhibitory effect on LABs. These results are in accordance with the previous studies (Turantas *et al.* 1999). But in case of M_1, M_2 and M_3 there was a significant decrease in microbial count

throughout storage study. The observations revealed that microwave treatment had a better control on microbial population as compared to sodium benzoate. These results are in agreement with the earlier study (Arenzana *et al.* 2012).

(b) Sensory quality

The overall acceptability of the samples are presented in the table 2. The score for S_1, S_2 and S_3 were 8.3, 8.1 and 8.6, respectively which decreased to 7.1, 6.9 and 6.4 respectively after 30 days of storage. The score of M_1, M_2 and M_3 decreased from 8.5, 8.6 and 7.9 to 8.1, 6.8 and 7.5, respectively on the 30 day of storage. These results show that the overall acceptability of the M_1 received highest rating from the panelists. The sensory quality of the samples that were treated with sodium benzoate did not differ significantly from that of control. However the sample M_2 received significantly lower scores from the panelists among the microwave treated samples. The samples M_2 and M_3 received the lower scores due to the textural degradation by microwave heating. These results are in agreement with the earlier study (Turantas *et al.* 1999).

Table 2: Sensory quality (Overall acceptability) of fermented cauliflower at different storage periods

Treatment	15 day	20 day	25 day	30 day
C	8.9±0.01^{a}	8.6±0.08^{d}	8.5±0.15^{g}	7.3±0.05^{b}
S_1	8.3±0.08^{f}	6.6±0.02^{b}	5.4±0.10^{d}	7.1±0.08a
S_2	8.1±0.15^{d}	7.7±0.02^{f}	7.2±0.04^{f}	6.9±0.15^{a}
S_3	8.6±0.02^{b}	7.9±0.01^{c}	7.4±0.05^{b}	6.4±0.03c
M_1	8.5±0.15^{g}	8.3±0.03^{g}	7.9±0.40^{e}	8.1±0.15^{d}
M_2	8.6±0.05^{b}	7.4±0.01^{c}	7.1±0.03^{g}	6.8±0.03e
M_3	7.9±0.02^{c}	7.6±0.05^{f}	7.2±0.04^{c}	7.5±0.04^{f}

Values are means ±SD and different letters indicates significant differences ($p<0.05$) among samples by Duncan's multiple range test.

(c) pH value

The results obtained for pH value are given in the Fig. 2. The pH value of the raw sample was 6.5 and was found to apparently decrease in different samples during storage. Several other authors have also reported such a decreasing trend in the pH values of the pickles during storage (Khaskheli *et al.* 2015; Turantas *et al.* 1999; Spiros *et al.* 2020; Dhanapal *et al*. 1994). In case of control (C) the pH value decreased from 5.74 to 3.70 on 30-day of fermentation. The same trend in the decrease of pH value in the garlic pickle has been earlier reported (Raja and Mir 2016). Sample M_1 showed decrease in the pH value from 5.62 at 15-day to 4.84 at the 30-day of fermentation. However the samples S_1, S_2 and S_3 did not showed any significant difference to that of control (C).

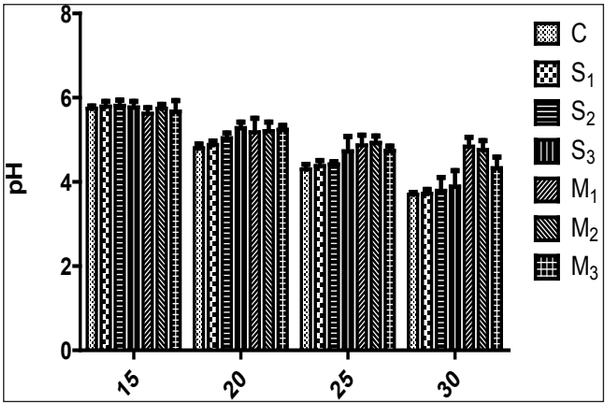

Fig. 2: pH of different samples of cauliflower pickle

(d) Titratable Acidity

The values of the titratable acidity obtained for the different samples are given in the table 3. The value of acidity (as % lactic acid) for the raw material was 0.27. The values of acidity for S_1, S_2 and S_3 were 0.90, 0.91 and 0.93, respectively on the 15-day of fermentation which increased to 1.45, 1.43 and 1.48 %, respectively at 30-day of storage, thus suggesting a significant increase in acidity. The samples M_1, M_2 and M_3 showed an decrease in the acidity values from 0.97, 0.99 and 0.92 on the 15-day of fermentation to 0.96, 0.97 and 0.86 respectively at 30-day of fermentation. Increase in acidity of M_1, M_2 and M_3 samples during storage might be due to release of H^+ and NH_3+ from putrifecation. It is thus evident from the results that microwave treatment had a better control on acid production than sodium benzoate and the effect can

be attributed to destruction of lactic acid bacteria by microwaves (Arenzana *et al.* 2012).

Table 3: Acidity (% lactic acid) value of fermented cauliflower at different storage periods

Treatment	15 day	20 day	25 day	30 day
C	0.89±0.03^{a}	0.92±0.05^{d}	1.2±0.05^{g}	1.45±0.21^{b}
S_1	0.90±0.08^{f}	0.96±0.02^{b}	1.34±0.10^{d}	1.43±0.03^{a}
S_2	0.91±0.05^{d}	0.95±0.05^{f}	1.37±0.14^{f}	1.48±0.13^{a}
S_3	0.93±0.03^{b}	0.96±0.11^{c}	0.99±0.15^{b}	1.12±0.06c
M_1	0.97±0.15^{g}	1.07±0.13^{g}	1.11±0.40^{e}	0.96±0.15^{d}
M_2	0.99±0.05^{b}	1.09±0.05^{c}	1.04±0.03^{g}	0.97±0.13^{e}
M_3	0.92±0.10^{c}	1.02±0.05^{f}	0.99±0.06^{c}	0.86±0.04^{f}

(e) Texture

The firmness (N) values obtained for control and samples treated with microwave and sodium benzoate are given in the table 4. All samples showed decrease in the firmness during storage. Sample S_2 showed the highest decline (4.9) at 30-day of fermentation. For the sample S_1, S_2 and S_3 the firmness decreased from 6.8, 6.9 and 6.9 to 5.3, 4.9 and 5.1, respectively after 30 days of storage. Samples M_1 (6.6) and M_2 (6.3) also show a decrease in the firmness after 30 days of storage. Similar trend were observed in pickled vegetables due to thermal processing (Fleming *et al.* 1993; Papageorge *et al.* 2003).

Table 4: Firmness (N) value of fermented cauliflower at different storage periods

Treatment	15 day	20 day	25 day	30 day
C	6.8±0.03^{a}	6.7±0.05^{d}	6.3±0.05^{g}	6.1±0.21^{b}
S_1	6.8±0.08^{f}	6.6±0.02^{b}	5.4±0.10^{d}	5.3±0.03^{a}
S_2	6.9±0.05^{d}	6.7±0.05^{f}	5.3±0.14^{f}	4.9±0.13^{a}
S_3	6.9±0.03^{b}	6.4±0.11^{c}	6.3±0.15^{b}	5.1±0.06c
M_1	7.0±0.15^{g}	6.8±0.13^{g}	6.7±0.40^{e}	6.6±0.15^{d}
M_2	7.0±0.05^{b}	7.1±0.05^{c}	6.7±0.03^{g}	6.3±0.13^{e}
M_3	6.9±0.10^{c}	6.7±0.05^{f}	6.5±0.06^{c}	6.2±0.04^{f}

Values are means ±SD and different letters indicates significant differences (p<0.05) among samples by Duncan's multiple range test.

(f) Color

The values obtained for the instrumental color of the samples during storage study are given in the table (5). The "L" value showed a constant decrease in the control sample (38.97 to 36.38) during the storage. However "L" value increased in the S_1 (39.32 to 41.22), S_2 (39.86 to 43.11) and S_3 (42.41 to 43.26) samples. The "a" value increased continuously among all treated samples including control sample from day 0 up to day 30 of the fermentation. The "b" value showed increasing trend in all samples including control. Similar trend in instrumental color was observed in carambola pickle (Igrid and Neela, 2004).

Table 5: Color value of fermented cauliflower at different storage periods

Sample	Attributes	15 days	20 days	25 days	30 days
	L	38.97	38.10	37.41	36.38
Control	*a*	-1.43	-1.22	1.43	1.63
	b	8.76	8.93	10.33	11.30
	L	39.32	39.94	40.31	41.22
S_1	*a*	-1.49	-1.58	1.43	1.98
	b	8.81	8.99	10.91	11.21
	L	39.86	39.98	41.12	43.11
S_2	*a*	-1.57	-1.63	1.73	2.14
	b	8.93	9.31	11.32	11.49
	L	42.41	40.06	41.68	43.26
S_3	*a*	-2.62	-2.86	2.01	2.32
	b	8.97	9.42	11.43	11.63
	L	46.23	42.23	42.99	44.18
M_1	*a*	-2.82	-2.91	2.22	2.68
	b	9.01	9.44	11.96	12.21
	L	52.31	42.46	43.68	44.88
M_2	*a*	-2.96	-3.02	3.15	3.43
	b	9.32	9.56	12.01	12.53
	L	58.68	42.66	43.81	44.96
M_3	*a*	-3.01	-3.33	3.43	3.53
	b	9.38	9.63	12.22	12.43

CONCLUSION

This study demonstrated the applicability of microwave treatment as an effective method to arrest/ slowdown the bacterial growth during fermentation.

While as application of sodium benzoate did not show any significant inhibitory effect on bacteria's particularly LABs at concentration of up to 550 ppm. However minimal texture degradation due to microwave heating needs to be overcome. Pre-treatments must be applied to improve the textural properties of the pickle. Also the microwave exposure for longer time had slight negative influence on the sensory attributes of the pickle. Therefore a proper technological intervention is needed to enhance the overall quality parameters of the fermented Cauliflower pickle.

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