

Research Article

An Improved Combination Treatment for Ensuring Safety and Extending Shelf Life of Sweet Corn Kernels

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Abstract

The shelled sweet corn kernel is prone to microbial and pathogenic contaminations primarily due to post-harvest handling which affect its safety and storage life. As an attempt to address this issue, a method was developed which included NaOCl wash (200 ppm; 5 min), hot water blanching (60°C; 5 min), and gamma irradiation (5 kGy). The treatments resulted in shelf life extension up to 30 days upon storage at 4°C. Beyond this storage, microbial load was found to be quite high with sharp declination in organoleptic rating. Now, the method has been further improved by replacing chlorination with other treatments such as thermosonication and antioxidant (cold ascorbate) dip. Besides, in the improved method required radiation dose was reduced to 2 kGy instead of 5 kGy. Thus, the improved method included thermosonication (34 kHz; 60°C; 7 min), cold ascorbate dip (4°C; 0.5%; 5 min), air drying (2 h), vacuum packaging (40%) and gamma irradiation (2 kGy) which extended the shelf life of sweet corn kernels up to 40 days when stored at 4°C. These samples were found to be free from presumptive coliform and *Staphylococcus* species and also low in microbial load. The quality attributes in terms of physical, nutritional, sensory and antioxidant properties were found to be well retained during storage. The study provides better combinations to ensure safety and extend shelf life of the shelled sweet corn kernels.

Keywords: Ascorbate dip; Gamma irradiation; Microbial safety; Thermosonication; Vacuum packaging

Introduction

The shelled sweet corn kernel is widely used in various ready-to-eat fast food preparations worldwide as salads, steamed corn, and soup [1,2]. The kernels are quite vulnerable to microbial and pathogenic contaminations due to high moisture and sugar contents

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and hence, cannot be considered safe for raw consumption [3]. Partial cooking/steaming may not completely ensure its safety. A recent study from this laboratory has reported the occurrence of presumptive coliforms in freshly procured shelled sweet corn kernels from the market [4]. The major source of contamination is post-harvest shelling and further handling which results in its poor storage life. To address these issues, some studies have been performed earlier. These included film-over-wrap in tray and polyolefin stretch films [5]; shrink wrapping, refrigeration, and gamma irradiation (upto 1 kGy) [6]; and packaging in perforated packets, cold (4°C) acclimatization for 5 days followed by storage at -1°C [7]. The shelf life of kernels could not be extended beyond 20 days by these treatments. Some of these treatments even could not seem to be effective in ensuring inactivation of pathogens. The data from the USA (~ 713 produce-related outbreaks between 1990 and 2005) and the UK (~ 88 outbreaks between 1996 and 2006) have reported the involvement of fresh agri-produce in foodborne illnesses [8].

Recently, a combination process was developed in this laboratory. The process involved chlorination (NaOCl; 200 ppm, 5 min), blanching (60°C, 5 min), packaging in Low Density Polyethylene (LDPE) packets and gamma irradiation (5 kGy). The process could extend the shelf life of kernels up to 30 days at 4°C. Although chlorination has been recommended as a sanitization treatment for different food applications by US FDA, its direct application in food has not been approved in European countries such as the Netherlands, Sweden, Germany, and Belgium [9]. The prime reason for this is the safety concerns associated with possible formation of chlorinated compounds such as trihalomethanes, chloramines, halo ketones, chloropicrins, and haloacetic acids, suspected to be potential carcinogens [10,11]. Hence, an utmost need was felt to improve the combination process by replacing chlorination with another physical sanitization treatment.

Ultrasonication treatment was explored as an alternative to the chlorination. Ultrasound refers to pressure waves with a frequency of ≥ 20 kHz. Higher-power ultrasound at lower frequencies (20 to 100 kHz), is referred to as "power ultrasound" and has the ability to cause cavitations, which has applications in food processing to inactivate microorganisms [12]. A major advantage of ultrasound is that the sound waves are generally considered safe and non-toxic [13]. In parallel, ultrasonication coupled with blanching (thermosonication) was also explored. As a part of combination treatment, the processed samples were stored at low temperature (4°C). Ascorbic acid treatment has several beneficial properties such as control of browning and discoloration in food samples due to antioxidant and pH lowering properties [14,15]. In the improved combination, effect of vacuum packaging was also examined as lack of O₂ in packages minimizes the radiation induced oxidative changes and storage associated quality deterioration [16]. As a final treatment, gamma irradiation was included in the process however, here the dose was reduced which may help in making the process more cost effective and abide the new food rule governing radiation processing approved by Government of India [17]. Irradiation is a non-thermal food

processing technology having wide range of applications and has been approved by many national/international organizations such as World Health Organization (WHO), Food and Agriculture Organization (FAO), International Atomic Energy Agency (IAEA), and codex alimentarius and Food Safety and Standards Authority of India (FSSAI) [18,19].

To sum up, in the current study, a popular sweet corn variety (sugar 75) was subjected to various treatments such as thermosonication, ascorbate dip, vacuum packaging, and gamma irradiation for ensuring safety and extending the shelf life. The treated samples were stored at low temperature (4°C) and periodically examined for microbial, sensory as well as physical, biochemical and antioxidant properties.

Materials and Methods

Procurement of sweet corn

Sweet corn (var. sugar 75) cobs were harvested from the farm in Mancher, Pune district, Maharashtra, India and supplied to the local market of Mumbai, Maharashtra within 2 days of harvest. Freshly shelled sweet corn kernels (10 kg) were procured as per the experimental need from the local market (Mumbai) and subjected to combination treatments within 3 h of shelling.

Chemicals

The chemicals used in the study were of analytical grade, procured from Sigma-Aldrich Inc., St. Louis, MO, USA. Commercial grade ascorbate was procured from Prabhat Chemicals, Bharuch, Gujarat. Microbiological media were purchased from Himedia Laboratories Ltd., Mumbai.

Treatment conditions

The ultrasonic treatment was performed using an ultrasonic processor (OU-23-SPL, Oscar, India; power: 500 W; frequency: 34 ± 3 kHz; transducer: PZT) equipped with digital temperature control. The samples were exposed to ultrasound for different time points (2.5, 4, 7, and 10 min) at 26, 50 and 60°C. On the basis of microbial and sensory analyses time and temperature of ultrasonication was optimized. Ascorbate (0.25, 5, and 1%) was solubilized in sterile water and the temperature of the solution was maintained at 4°C. The kernel was dipped in the cold ascorbate for different time points (2.5, 5, and 10 min). The treated samples were air dried in hygienic and aseptic condition for 2 h as standardized in our earlier study [4]. Later, the samples were vacuum packed (30, 45, and 60%) in sterile LDPE (175 gauge, permeability of CO₂: ~17,600 ml/m³/day and O₂: ~3050 ml/m³/day) packets and gamma irradiated at 2 kGy in a cobalt - 60 food package irradiator (AECL, Ottawa, Canada; dose rate: 40 Gy/min) at this institute [4]. Dosimetry was performed using a standard ceric-cerous (3 mM) dosimeter [20]. Dose uniformity or overdose ration (= Dose_{max}/Dose_{min}) was calculated as 1.3. The combination processed and unprocessed samples were stored at similar condition at 4°C and Relative Humidity (RH) of 99%.

Microbiological analysis

It was performed as per the Bacteriological Analytical Manual (BAM) [21]. In brief, 20 g of kernel was homogenized in 100 ml of saline (0.85%) under aseptic conditions and serially diluted in the same. For Aerobic Plate Counts (APC) dilutions were pour plated on Plate Count Agar (PCA) and incubated at 30°C for 2 days. For Aerobic Spore Counts (ASC) and Anaerobic Spore Counts (AnSC),

sample homogenate (1 ml) was heated (80°C for 10 min) in a water bath, serially diluted, and pour-plated on tryptic soya and reinforced clostridial agar plates and incubated at 37°C for 2 and 4 days in aerobic or anaerobic conditions, respectively. For Yeast and Mould Counts (YMC), the homogenate (100 µl) was spread plated on rose-bengal chloramphenicol agar plates and incubated at 25°C for 5 days. Presumptive Coliforms (PC) and *Staphylococcus* species (PS) were enumerated in violet red bile agar and Baird Parker agar base with egg yolk tellurite emulsion (5%) plates, respectively.

Analysis of physical qualities

Moisture content (%) was determined by infrared based moisture analyzer (Sartorius MA 100, Chicago, USA). Texture analysis was carried out using TA - HD plus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK), equipped with a 75 mm aluminum platen compression probe (P/75) as discussed earlier [4]. The color of the samples was assessed by a colorimeter (Konica Minolta Sensing Inc., CM-3600d, Osaka, Japan) using JAYPAK 4808 software (quality control system, Version 1.2) as discussed earlier [4]. Color values were expressed in terms of LAB parameters ['L' (Lightness), '+a' (redness) or '-a' (greenness) and '+b' (yellowness) or '-b' (blueness)] and also in terms of 'C' (chroma) and 'h' (hue angle).

Analysis of biochemical properties and antioxidant capacity

Samples were ground with liquid nitrogen using mixer-grinder, lyophilized (Alpha 2-4 Freeze Dryer, Martin Christ, Osterode, Germany) and stored at -70°C. The powder (5 g) was suspended in 100 ml of milli Q water, mixed using vortex for 30 s and centrifuged (15,000 x g, 20 min). The supernatant was filtered (0.22 µm filter) and used for analyses of biochemical properties and antioxidant capacity. The total and reducing sugars were determined using dinitrosalicylic acid based on the standard curves of sucrose and glucose solutions, respectively [22]. Prior to total sugars analysis, the sample was treated with HCl (2N, 68°C, 8 min) for inversion of sucrose to reducing sugars, cooled to ambient temperature, and neutralized by addition of NaOH. The total soluble phenolics were determined by Folin-Ciocalteu colorimetric method and expressed as Gallic Acid Equivalents (GAE) as described earlier [23]. The flavonoid content was determined by aluminium chloride colorimetric method and expressed as Catechin Equivalents (CAE) [23]. The outcome of biochemical analyses was expressed with respect to the fresh weight of shelled kernels. Antioxidant capacity was determined using 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) and 2,2'-Azino-Bis-3-ethylbenzthiazoline-6-Sulphonic acid (ABTS) radical-scavenging assays as described earlier [23,24].

Organoleptic evaluation

The shelled sweet corn kernels (250 g) were cooked in a pressure cooker for 10 min and served to panelists (25 nos.) in a taste panel laboratory in individually partitioned compartments for sensory analysis [25]. The quality parameters (appearance, color, texture, odor, taste, and overall acceptability) were evaluated on a 9-point hedonic scale (9 = 'like extremely', 8 = 'like strongly', 7 = 'like very well', 6 = 'like fairly well', 5 = 'like moderately', 4 = 'like slightly', 3 = 'dislike slightly', 2 = 'dislike moderately' and 1 = 'dislike extremely').

Analysis of genotoxic safety of the processed product

It was performed by determining the mutagenic potential of the processed product using *E. coli* MG1655 cells. This assay is based upon RNA polymerase (*rpoB*) associated rifampicin resistance (Rif^R)

Treatments	Storage Period (days)	APC ¹	ASC ²	AnSC ³	PC ⁴	PS ⁵	YMC ⁶
Fresh (Control)	0	8.6 ± 0.06 ⁱ	2.3 ± 0.04	3.1 ± 0.02 ⁱ	4.5 ± 0.03 ⁱ	5.1 ± 0.05 ⁱ	6.2 ± 0.09 ⁱ
Ultrasonication At ambient temp (26 ± 2°C; 7 min)	0	6.2 ± 0.07 ^u	ND*	1.3 ± 0.01 ^u	4.2 ± 0.04 ⁱ	3.6 ± 0.05 ^u	4.3 ± 0.03 ^u
At blanching temp. (60°C; 7 min)	0	2.3 ± 0.02 ^v	ND	ND	ND	ND	ND
Irradiation (2 kGy)	0	4.8 ± 0.03 ^w	ND	ND	ND	ND	3.6 ± 0.04 ^v
Ultrasonication (60°C; 7 min) and Irradiation (2 kGy)	0	ND	ND	ND	ND	ND	ND
Processed ^d	0	ND	ND	ND	ND	ND	ND
	35	2.1 ± 0.01 ^v	ND	ND	ND	ND	ND
	40	3.1 ± 0.02 ^w	ND	ND	ND	ND	ND
	45	5.1 ± 0.04 ^w	ND	ND	ND	ND	ND

Table 1: Microbial load (log cfu/g) in unprocessed and processed sweet corn kernels during storage.

¹Aerobic plate counts; ²Aerobic spore counts; ³Anaerobic spore counts; ⁴Presumptive coliforms; ⁵Presumptive *Staphylococcus species*; ⁶Yeast and mould counts; *Not detectable (detection limit 10 cfu/g); ^uUltrasonication (34 kHz; 60°C; 7 min), cold ascorbate (0.5%) dip (4°C, 5 min), air drying (2 h), vacuum packaging (40%) and irradiation (2 kGy); Different letters column-wise (t-y) indicate significant differences among means (P ≤ 0.05)

phenotype as described earlier [26,27]. Mutation frequency was calculated as the ratio of total no. of Rif^R mutants to the total no. of viable cells per ml. Spontaneous mutation frequency was also determined for cells grown similarly in standard growth (LB broth) medium. Ethyl Methanesulfonate (EMS: 133 mM), a known chemical mutagens, was used as a positive control.

Statistical analyses

All the experiments were performed in three independent sets, each set having at least three replicates. One-way ANOVA ($\alpha = 0.05$) was performed taking all the data using BioStat 2009 Professional 5.8.0.0 software (AnalystSoft Inc., Vancouver, BC, Canada).

Results and Discussion

Microbiological quality of fresh shelled kernels

The fresh sweet corn cob (unshelled) when aseptically cut into pieces and subjected to microbial analysis indicated significant microbial load. The APC was found to be ~ 4.7 log cfu/g, whereas ASC was ~ 0.3 log cfu/g, AnSC was ~ 0.3 log cfu/g, PC was ~ 3.0 log cfu/g, PS was ~ 2.2 log cfu/g, and YMC was 2.7 log cfu/g (data not shown). However, shelled sweet corn samples procured from market were found to have very high microbial load including APC: ~ 8.5 log cfu/g, ASC: 2.3 log cfu/g, AnSC: 3 log cfu/g, PC: 4.5 log cfu/g, PS: 5 log cfu/g, and YMC: 6 log cfu/g (Table 1). This indicated significant contribution of shelling practice in the increased microbial load. Similar findings have been reported earlier too from this laboratory [4]. In that study, presumptive coliform were biochemically characterized by IMViC test where different coliform biotypes '- - + + (32%)' and '+ + - - (5%)' typical for *E. coli* and *Enterobacter aerogenes*, respectively, were observed.

The results of microbial and presumptive pathogenic load indicated unsafe nature of shelled sweet corn kernels procured from the market for direct (raw) consumption in various preparations. Such contamination was also reported in other fresh produces including sprouts, minimally processed vegetables, and leafy vegetables [28]. Hence, there is an utmost need to develop a safe treatment protocol to hygienize the produce like sweet corn kernels and also to extend its shelf life to control post harvest losses.

Evaluation of different processing methods to achieve hygienization with retained sensory quality of shelled sweet corn kernels

Various processing methods were evaluated either alone or in combination for their efficacy to reduce microbial load while maintaining the sensory qualities as primary criteria for the optimization of the treatments.

Treatments for microbial decontamination

Effect of ultrasonication: Ultrasonication was performed at two temperatures, ambient (26°C) and blanching (60°C). The treatment timing selected was 7 min. The treatment time for 10 min was found to negatively affect textural quality whereas time lesser than 7 min was found to be low in hygienization efficacy (data not shown). Ultrasonication at ambient temperature for 7 min reduced ASC to undetectable level (< 10 cfu/g) and APC, AnSC or PS counts by ~2 log cfu/g (Table 1). Interestingly, PC was not found to reduce significantly (Table 1). In another study too, *E. coli* was not found to be very sensitive to ultrasonic treatments at ambient temperature [29].

Thermosonication (60°C) was optimized through a preliminary trial study to achieve maximum microbial inactivation without affecting sensory qualities such as textural and discoloration of samples (data not shown). Ultrasonication treatment at the blanching temperature of 60°C for 7 min was found to reduce APC by 6 log cfu/g and ASC, AnSC, PC, PS, and YMC counts to below detectable level (Table 1). In other study too, thermosonication was found to be more effective than blanching (65°C) alone in reducing microbial load from red bell peppers, strawberries and watercress [30]. Thus, in the current study, thermosonication was preferred for sample processing instead of sonication alone.

Effect of gamma radiation: As per the new food rule pertaining to radiation processing approved by the Government of India, fresh fruit and vegetable products belong to class 2 of generic classification category and minimum and maximum dose for their shelf life extension have been set at 1 and 2.5 kGy, respectively [17]. Hence, gamma radiation at average absorbed dose of 2 kGy was evaluated for its efficacy to hygienize the shelled sweet corn kernels. It reduced the APC and YMC by 4 and 3 log cfu/g, respectively. In the radiation treated samples, ASC, AnSC, PC and PS were below detection level. Gamma radiation treatment has been well proven technology to reduce microbial load in food samples [23].

Further, combinations of thermosonation (60°C, 7 min) or gamma radiation (2 kGy) were explored to achieve the best possible level of hygienization (preferably < 10 cfu/g). This will help in extending the shelf life of the processed kernels up to the maximum period and thus reduce the post harvest losses. The combination of thermosonation and gamma radiation reduced the microbial load to below detectable level (Table 1).

Treatments for retention of organoleptic quality: Besides achieving hygienization, retention of the sensory qualities during storage is also equally important criteria for deciding the optimal combination treatments. For this, hygienized kernels were subjected to an antioxidant treatment by dipping in ascorbate solution at two conditions, ambient as well as cold temperature. Also, vacuum packaging of the processed produce was assessed for retaining the sensory attributes.

Effect of ascorbate dip: The thermosonicated samples were dipped in cold ascorbate (4°C, 0.5%) which was found to prevent discoloration during storage and thus helped in retaining its visual appeal (data not shown). The cold treatment has earlier been reported in reducing the over blanching effect of the product [31]. Ascorbate at the concentration > 0.5% was not found to further improve the shelf life and appeal of the product (data not shown). Ascorbate at 0.5% concentration has also been reported earlier for prevention of egg plant discoloration [32].

Effect of vacuum packaging: The above treated (thermosonicated and cold ascorbate dipped) samples were air dried under aseptic condition for 2 h and vacuum (40%) packed to extend the life by reducing radiation induced or storage associated oxidative damage and inhibiting growth of microbes [33]. Vacuum packaging at 40% was found to be optimal for the produce. At further higher level of vacuum (50 or 60%), significant change in texture was observed in the samples (data not shown).

Optimized processing protocol

The processing combination optimized for treating shelled sweet corn kernels included a) thermosonation (60°C; 7 min), b) cold ascorbate (0.5%) dip (4°C), c) air drying (2 h), d) vacuum packaging (40%) and e) gamma radiation (2 kGy). The order of these five steps of processing kept fixed to achieve the desired objective. Changes in the order did not suffice the purpose in the effective manner (data not shown).

Cold water (sterile) instead of cold ascorbate (0.5%) dip was not found to be very effective. Similarly, ascorbate (0.5%) dip at ambient temperature was also not found to be effective. These changes were found to be critical as gradual discoloration in these samples observed during storage (data not shown). Organoleptically, these samples were acceptable only up to 25-30 days (data not shown).

Quality evaluation of processed samples during storage

The optimized combination processed samples were analyzed for various quality parameters periodically up to 45 days, whereas control samples could be analyzed only up to 15 days. After this, visible microbial growth was observed in control samples. Organoleptic quality of control was assessed up to 2 days, as microbial and pathogenic load reached to higher level, making the product unsafe during storage.

Microbiological quality: Microbial load in the processed sample at day 0 (the day of start of the experiment) was found to be below

detectable level and APC level reached up to 3 and 5 log cfu/g in processed samples on day 40 and 45, respectively (Table 1). However, other microbial loads and presumptive pathogenic loads were still below detectable level (Table 1). Several earlier studies have shown the advantage of vacuum packaging and gamma radiation in shelf life extension of food [34,35]. The processed kernels having microbial load up to 3 log cfu/g can be considered safe as pathogenic microbes were not detected. Similar guidelines has been set by United States Department of Agriculture (USDA), where high microbial load in fresh produce such as fruits and vegetables was not considered to be the rejection criteria as long as it does not have pathogenic load [36]. As per UNIDO (United Nations Industrial Development Organization), the hygiene level of ready-to-eat processed food in terms of total aerobic bacteria should be < 4 log cfu/g; fungi and *Enterobacter* and other gram negative organisms should be < 2 log cfu/g, whereas count of *E. coli* should be nil [37].

Physico-chemical properties and antioxidant capacity: The moisture content in the control kernels was found to be ~ 73% which significantly reduced during 15 days of storage to ~ 65%, however no significant change was observed in case of processed samples (Table 2). Similarly, significant loss in the texture was observed in the control samples during storage of 15 days, whereas not in case of processed samples even on 45 days of storage (Table 2). The color parameters such as 'L', 'a', 'b', 'C' and 'h' in the control samples were ~ 82, 5, 28, 27 and 80, respectively and not found to be affected due to processing (Table 2). The cold ascorbate dip followed by vacuum packaging seem to be quite helpful in the retention of natural color in sweet corn during prolonged storage as reported in an earlier study, where ascorbate (0.5%) in combination with vacuum infiltration was reported to be more effective in preventing discoloration of plum during storage [38].

In the processed samples, reducing sugar (~ 0.85 %), total sugars (~ 1.8 %), total soluble phenolic (0.27 mg GAE/g), and flavonoids (0.01 mg CAE/g) were found to be similar to fresh control and retained up to 40 days of storage (Figure 1). This could be attributed to combination effect of treatments used in the current study leading to reduced physiological changes during storage [18,31]. Corn possesses many phytochemicals such as phenolics and flavonoids, which may contribute to its antioxidant properties [39]. Thus, effect of processing on antioxidant property was assessed by analyzing DPPH and ABTS radical-scavenging activities in the processed samples. These were found to be ~37 and ~48%, respectively, similar to control kernels and retained up to 40 days (Figure 2). This could be attributed to retention of phytochemicals including phenolics in these samples [23]. A sudden decrease in most of the quality parameters in the processed samples was observed on day 45 (Figures 1 and 2), mostly due to activation of natural senescence due to physiological changes [40].

Organoleptic properties: Overall acceptability of the control sample on a 9 point hedonic scale was rated to 7.5 which after 2 days reduced to 4.3 (Table 3). In the processed sample, the overall acceptability was 7.2 even after 40 days of storage (Table 3). Such higher rating could be due to better retention of sugars and control of yeasts and molds in the processed samples leading to inhibition of the formation of undesirable fermentative off-flavor during storage [4].

Parameters	Unprocessed		Processed [#]		
	0 d	15 d	0 d	40 d	45 d
Moisture (%)	73 ± 2 ^a	65 ± 2 ^b	74 ± 2 ^a	74 ± 1 ^a	74 ± 1 ^a
Texture (g)	31 ± 3 ^a	23 ± 4 ^b	30 ± 3 ^a	32 ± 4 ^a	32 ± 6 ^a
Color					
'L' value	81.2 ± 3.6 ^a	83.0 ± 2.1 ^a	81.8 ± 2.1 ^a	79.4 ± 1.5 ^a	80.7 ± 1.4 ^a
'a' value	5.4 ± 1.5 ^a	4.6 ± 2.3 ^a	5.0 ± 0.7 ^a	5.5 ± 1.1 ^a	3.0 ± 1.1 ^b
'b' value	28.4 ± 6.4 ^a	28.9 ± 4.7 ^a	8.5 ± 1.9 ^a	30.0 ± 2.5 ^a	28.7 ± 2.6 ^a
'C'	26.8 ± 6.6 ^a	28.8 ± 5.2 ^a	28.9 ± 2.0 ^a	30.4 ± 2.4 ^a	28.9 ± 2.8 ^a
'h'	79.7 ± 2.2 ^a	81.1 ± 3.3 ^a	80.0 ± 1.8 ^a	80.2 ± 1.5 ^a	83.2 ± 1.7 ^b

Table 2: Effect of combination processing on the physical properties of sweet corn kernels.

[#]As detailed in table 1, Different letters row-wise (a,b) indicate significant differences among means ($P \leq 0.05$)

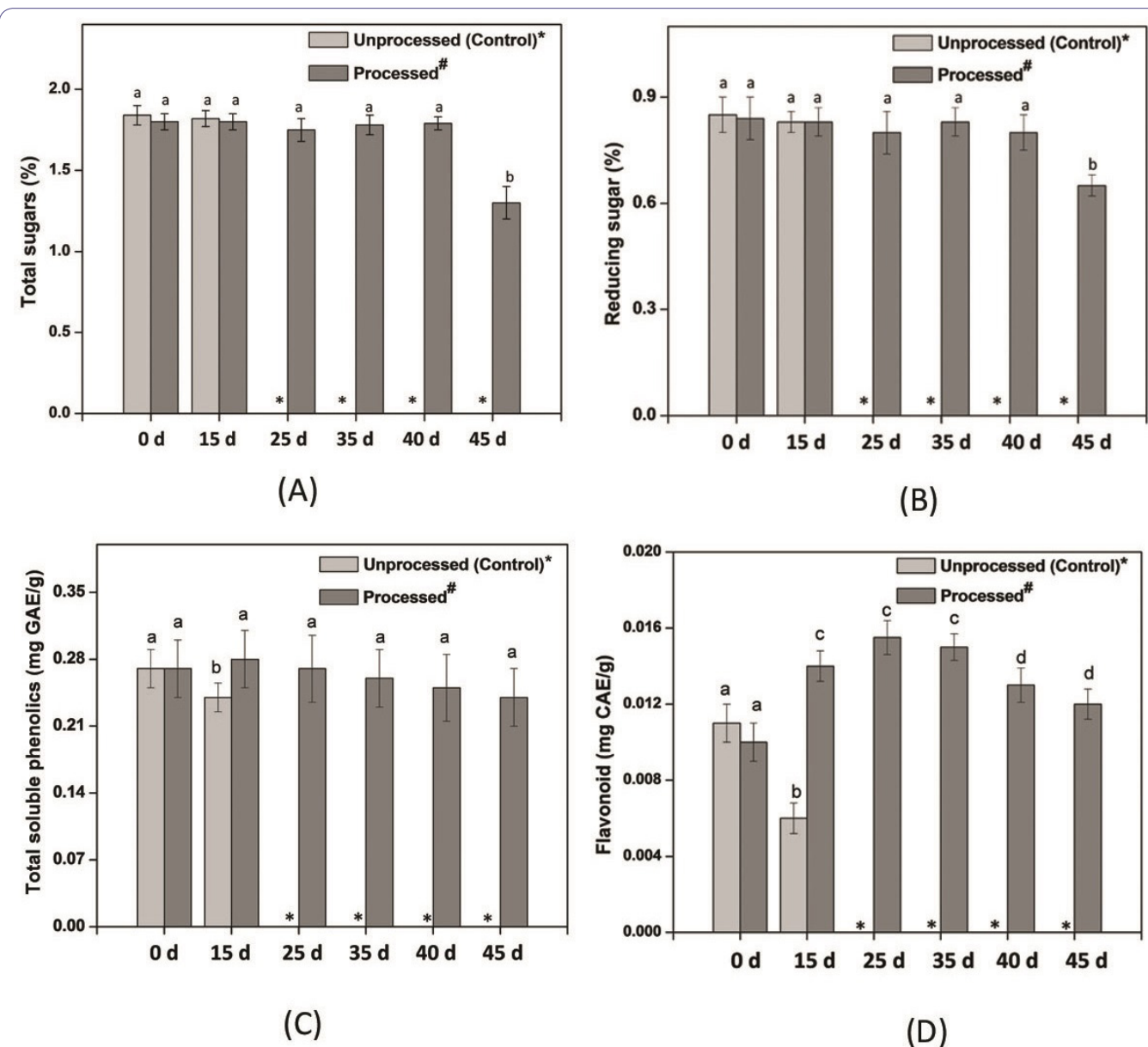


Figure 1: Biochemical properties of processed[#] sweet corn kernels during storage.

(A) Total sugars, (B) Reducing sugar, (C) Total soluble phenolics, and (D) Flavonoid content; [#]As detailed in table 1; *Unprocessed control samples could be analyzed only up to 15 days due to visual microbial growth beyond this period; Different letters (a-d) indicate significant differences among means ($P \leq 0.05$)

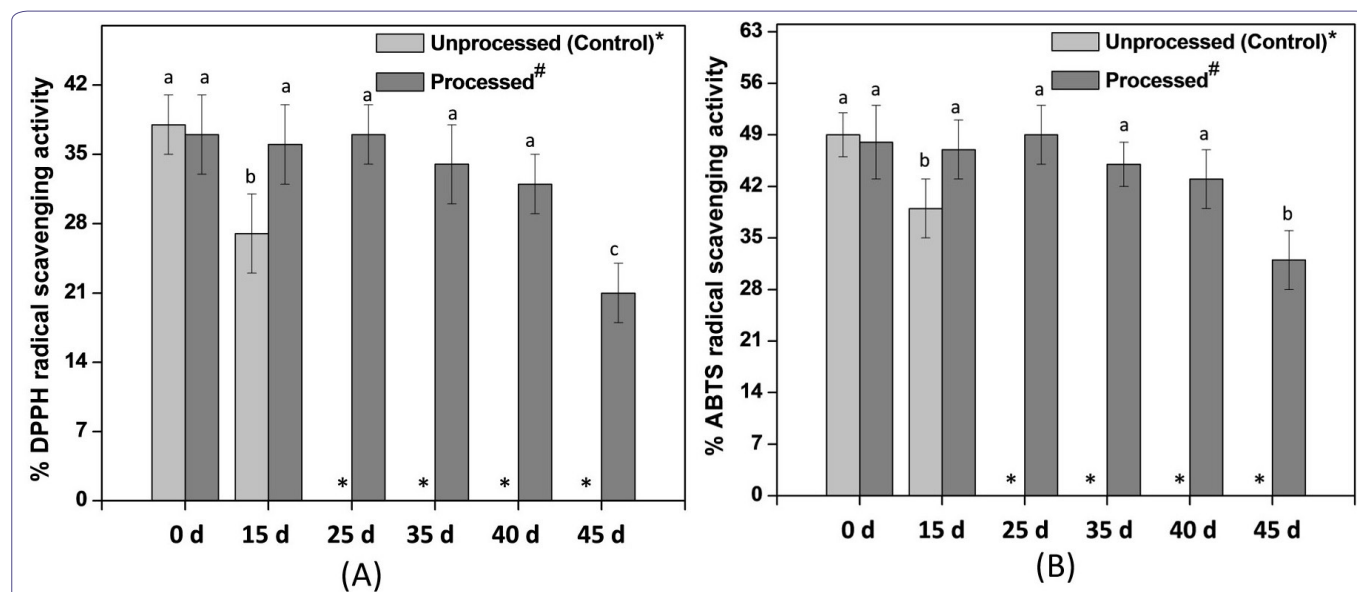


Figure 2: Antioxidant capacity of combination processed sweet corn during storage.

(A) DPPH and (B) ABTS radical scavenging activity; *As detailed in table 1, *Unprocessed control samples could be analyzed only up to 15 days due to visual microbial growth beyond this period; Different letters (a-c) indicate significant differences among means ($P \leq 0.05$)

Sensory attributes	Unprocessed		Processed#	
	0 d	2 d	0 d	40 d
Appearance	7.9 ± 1.0 ^{a,t}	7.5 ± 1.0 ^{a,t}	7.8 ± 0.9 ^{a,t}	7.5 ± 1.0 ^{a,t}
Aroma	7.6 ± 1.2 ^{a,t}	4.3 ± 1.1 ^{b,u}	7.6 ± 1.1 ^{a,t}	7.6 ± 0.9 ^{a,t}
Taste	7.5 ± 0.9 ^{a,t}	5.2 ± 0.7 ^{b,u}	7.5 ± 1.2 ^{a,t}	7.3 ± 1.1 ^{a,t}
Texture	7.2 ± 1.1 ^{a,t}	6.1 ± 0.6 ^{a,t}	7.1 ± 1.2 ^{a,t}	7.2 ± 0.8 ^{a,t}
Overall acceptability	7.5 ± 0.9 ^{a,t}	4.6 ± 0.9 ^{b,u}	7.4 ± 1.1 ^{a,t}	7.2 ± 1.1 ^{a,t}

Table 3: Effect of combination processing on sensory quality* of sweet corn kernels.

*As detailed in Table 1, *9 point hedonic scale: 9 = 'like extremely', 8 = 'like strongly', 7 = 'like very well', 6 = 'like fairly well', 5 = 'like moderately', 4 = 'like slightly', 3 = 'dislike slightly', 2 = 'dislike moderately' and 1 = 'dislike extremely'; Different letters row-wise (a,b) and column-wise (t,u) indicate significant differences among means ($P \leq 0.05$)

Genotoxic safety assessment

As different combinations were used in this study, the genotoxic safety assessment of the processed product was also performed. The genotoxicity was analyzed in wild type *E. coli* MG1655 cells grown exclusively on medium prepared from processed kernels using *rpoB* gene based rifampicin sensitive to resistant (Rif^S→Rif^R) assay [26,27]. The results did not indicate any genotoxic effect whereas in case of *E. coli* cells treated with EMS (positive control), mutation frequency was found to be ~2700 cfu/10⁸ cells (data not shown). Similar findings have been observed in our earlier study [4].

Budget evaluation for optimized process

At laboratory scale, the optimum combination process could cost approximately rupees (Rs.) ~ 6.5 (~ 0.1 \$) (thermosonication: Rs. 0.24; ascorbate: Rs. 3; irradiation: Rs. 3), whereas our earlier method cost ~ Rs. 8 (~ 0.12 \$) (chlorination: Rs. 0.3; irradiation: Rs. 7.5) for 1 kg. The cost of processing can be further minimized in industrial scale. Thus, the current processing method was found to be comparatively cost effective.

Conclusion

A process including thermosonication, cold ascorbate dip, air drying, vacuum packaging in LDPE and gamma radiation (2 kGy)

was found to ensure microbiological safety and extend the shelf life of freshly shelled sweet corn kernels up to 40 days at 4°C. Thus, developed method assured better shelf life than our earlier reported combinations where shelf life up to 30 days was achieved. The quality attributes in terms of physical, nutritional, sensory, and antioxidant properties of combination processed kernels were also well retained during storage.

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References

- Esena RK, Owusu E (2013) Quality of cooked foods in urban schools in Ghana: Review of food borne diseases and health implications. International Journal of Scientific & Technology Research 2: 267-275.
- López-Martínez LX, Parkin KL, García HS (2012) Effect of processing of corn for production of masa, tortillas and tortilla chips on the scavenging capacity of reactive nitrogen species. Int J Food Sci Technol 47: 1321-1327.
- Suleiman RA, Rosentrater KA, Bern CJ (2013) Effects of deterioration parameters on storage of maize: A review. Journal of Natural Sciences Research 3: 147-165.

4. Kumar S, Gautam S, Sharma A (2015) Hurdle technology including chlorination, blanching, packaging and irradiation to ensure safety and extend shelf life of shelled sweet corn kernels. *Journal of Food Processing and Preservation* 39: 2340-2347.
5. Aharoni Y, Copel A, Gil M, Fallik E (1996) Polyolefin stretch films maintain the quality of sweet corn during storage and shelf-life. *Postharvest Biology and Technology* 7: 171-176.
6. Deák T, Heaton EK, Hung YC, Beuchat LR (1987) Extending the shelf life of fresh sweet corn by shrink-wrapping, refrigeration, and irradiation. *Journal of Food Science* 52: 1625-1631.
7. Shao X, Li Y (2011) Quality control of fresh sweet corn in controlled freezing-point storage. *African Journal of Biotechnology* 10: 14534-14542.
8. Goodburn C, Wallace CA (2013) The microbiological efficacy of decontamination methodologies for fresh produce: A review. *Food Control* 32: 418-427.
9. US Food and Drug Administration (2013) Microbiological safety evaluations and recommendations on sprouted seed. US Food and Drug Administration, Maryland, USA.
10. Bachelli ML, Amaral RD, Benedetti BC (2014) Alternative sanitization methods for minimally processed lettuce in comparison to sodium hypochlorite. *Braz J Microbiol* 44: 673-678.
11. Ölmez H, Kretzschmar U (2009) Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT-Food Sci Technol* 42: 686-693.
12. Piyasena P, Mohareb E, McKellar RC (2003) Inactivation of microbes using ultrasound: a review. *Int J Food Microbiol* 87: 207-216.
13. Kentish S, Ashokkumar M (2011) The physical and chemical effects of ultrasound. In: Feng H, Barbosa-Cánovas, Weiss J (eds.). *Ultrasound Technologies for Food and Bioprocessing*. Springer, London, UK. Pg no: 1-12.
14. Garcia E, Barrett DM (2002) Preservative treatments for fresh-cut fruits and vegetables. In: Lamikanra O (ed.). *Fresh-cut fruits and vegetables: Science, technology and market*. CRC Press, Boca Raton, Florida, USA.
15. Cortez-Vega WR, Becerra-Prado AM, Soares JM, Fonseca GG (2008) Effect of L-ascorbic acid and sodium metabisulfite in the inhibition of the enzymatic browning of minimally processed apple. *International Journal of Agricultural Research* 3: 196-201.
16. Zhou GH, Xu XL, Liu Y (2010) Preservation technologies for fresh meat - a review. *Meat Sci* 86: 119-128.
17. Food Safety and Standards Authority of India (2012) Draft Food Safety and Standards (Packaging and Labelling) & (Food Products Standards and Food Additives) (Amendment) Regulations, 2015 related to inclusion of New Atomic Energy (Radiation Processing of Food and Allied Products), Rules, 2012. Food Safety and Standards Authority of India, New Delhi, India.
18. Diehl JF (1995) Safety of irradiated foods. Marcel Dekker Inc., New York, USA.
19. Roberts PB (2014) Food irradiation is safe: Half a century of studies. *Radiation Physics and Chemistry* 105: 78-82.
20. Kumar S, Mishra BB, Saxena S, Bandyopadhyay N, More V, et al. (2012) Inhibition of pericarp browning and shelf life extension of litchi by combination dip treatment and radiation processing. *Food Chemistry* 131: 1223-1232.
21. US Food and Drug Administration (1988) Bacteriological analytical manual. US Food and Drug Administration, Arlington, Virginia, USA.
22. Kumar S, Khade HD, Dhokane VS, Behere AG, Sharma A (2007) Irradiation in combination with higher storage temperatures maintains chip-making quality of potato. *J Food Sci* 72: 402-406.
23. Kumar S, Gautam S, Powar S, Sharma A (2010) Microbial decontamination of medicinally important herbals using gamma radiation and their biochemical characterisation. *Food Chemistry* 119: 328-335.
24. Arnao MB, Cano A, Acosta M (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry* 73: 239-244.
25. Hajare SN, Gautam S, Nair AB, Sharma A (2014) Formulation of a nasogastric liquid feed and shelf-life extension using gamma radiation. *J Food Prot* 77: 1308-1316.
26. Kumar S, Gautam S, Sharma A (2013) Antimutagenic and antioxidant properties of plumbagin and other naphthoquinones. *Mutat Res* 755: 30-41.
27. Kumar S, Gautam S, Sharma A (2013) Identification of antimutagenic properties of anthocyanins and other polyphenols from rose (*Rosa centifolia*) petals and tea. *J Food Sci* 78: 948-954.
28. Jeddi MZ, Yunesian M, Gorji ME, Noori N, Pourmand MR, et al. (2014) Microbial evaluation of fresh, minimally-processed vegetables and bagged sprouts from chain supermarkets. *J Health Popul Nutr* 32: 391-399.
29. Alighourchi H, Barzegar M, Sahari MA, Abbasi S (2014) The effects of sonication and gamma irradiation on the inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* in pomegranate juice. *Iran J Microbiol* 6: 51-58.
30. Alexandre EMC, Santos-Pedro DM, Brandão TRS, Silva CLM (2011) Study on thermosonication and ultraviolet radiation processes as an alternative to blanching for some fruits and vegetables. *Food and Bioprocess Technology* 4: 1012-1019.
31. De Corcuera JIR, Cavalieri RP, Powers JR (2004) Blanching of foods. In: Heldman DR (ed.). *Encyclopedia of agricultural, food, and biological engineering*. Marcel Dekker Inc., New York, USA.
32. Barbagallo RN, Chisari M, Patanè C (2012) Use *in vivo* of natural anti-browning agents against polyphenol oxidase activity in minimally processed eggplant. *Chem Eng Trans* 27: 1-6.
33. Gould GW (1996) Methods for preservation and extension of shelf life. *Int J Food Microbiol* 33: 51-64.
34. Jouki M, Yazdi FT (2014) The effect of gamma irradiation and vacuum packaging upon selected quality traits of refrigerated ostrich meat. Part 2. Colour, texture and lipid oxidation properties. *Animal Science Papers and Reports* 32: 161-171.
35. Mantilla SPS, Santos EB, Vital HC, Mano SB, Freitas MQ, et al. (2011) Microbiology, sensory evaluation and shelf life of irradiated chicken breast fillets stored in air or vacuum. *Braz Arch Biol Technol* 54: 569-576.
36. United States Department of Agriculture (2014) Microbiological standards and guidelines. United States Department of Agriculture, Beltsville, Maryland, USA.
37. United Nations Industrial Development Organization (1984) Guidelines for commercial plantation and manufacture of medicinal and aromatic plants. United Nations Industrial Development Organization, Vienna, Austria.
38. Shao Y, Luo Y, Chen A, Chu H, Lu C, et al. (2011) Effects of a vacuum infiltration-based method with ascorbic acid on internal browning of plum (*Prunus salicina* lindell cv. Yuhuang) during cold storage. *Journal of Food Processing and Preservation* 35: 581-586.
39. Luo Y, Wang Q (2012) Bioactive compounds in corn. In: Yu L, Tsao R, Shihida F (eds.). *Cereals and pulses: nutraceutical properties and health benefits*. John Wiley & Sons, Hoboken, NJ, USA.
40. Riad GS, Brecht JK (2001) Fresh-cut sweet corn kernels. *Proc Fla State Hort Soc* 114: 160-163.