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Protocol development for minimally processed pomegranate arils

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SUMMARY :

Minimally processed pomegranate arils can be prepared by surface sanitization of fruits using 30ppm sodium hypochlorite for 15 minutes followed by rind removal, treating the arils with 1per cent calcium chloride for 10 minutes and refrigerated storage after packaging in aluminium tray covered with cling film. Samples were acceptable to the sensory panel even at the end of 5^{th} day.

KEY WORDS : Minimal processing, Pomegranate arils, Sodium hypochlorite, Calcium chloride, Packaging

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Consumer demand for high quality foods requiring only minimum effort and time for preparation has led to the introduction of ready-to-use fresh- cut or minimally processed products. Unfortunately short shelf-life is the major challenge faced; they undergo enzymatic browning, texture decay and microbial contamination, highly reducing their shelf-life. The increasing demand of these minimally processed produce represents a challenge for researchers and processors to make them stable and safe. Any processing technology for enhancing the convenience of consumer should definitely not affect the quality and safety of the produce. Many of the sophisticated technologies developed for enhanced shelf-life of fresh cut produce may not be economically feasible for adoption in developing countries like India. By establishing an efficient and economic protocol for development of minimally processed fruits, especially those which are difficult to prepare before consumption, consumers will be able to buy fresh fruits in ready to use form. Hence, an experiment was conducted at the Department of Processing Technology, College of Agriculture, Vellayani to standardize an efficient and economic protocol for development of minimally processed pomegranate arils with extended shelf-life and to study the acceptability of the standardized technology.

EXPERIMENTAL METHODS

The experiment was conducted in four continuous steps so as to develop a protocol for minimally processed pomegranate aril preparation, *viz.*, Surface sanitization, Evaluation of different pre-storage treatments, Development of packaging system and testing the acceptability of the standardized protocol.

Surface sanitization :

Good quality pomegranate fruits, procured from local market, were dipped in different sanitizing solutions *viz.*, 40^{o}C water, 30, 60, 90 and 120 ppm sodium hypochlorite for 15 minutes for surface de-contamination. Solutions were drained after treatment; 1cm^2 area of skin of the sanitized fruit was separated from fruit using a sharp sterilized blade and the treated pieces were evaluated for total microbial count using serial dilution spread plate technique. In order to avoid cross-contamination during sample preparation, knives, cutting boards and other equipments coming in contact with fruits were sanitized by immersing in 1000 ppm sodium hypochlorite solution for 10 minutes. Analysis of Co- variance was conducted for assessing the post treatment effect using pre-treatment as the covariate and the most effective sanitizing solution was determined based on the efficiency in controlling the micro-organisms.

Evaluation of different pre-storage treatments :

Pomegranate fruit was surface sanitized using the best method selected under part (a) of the experiment, Rind of the sanitized fruits was removed; inedible portions removed and arils were extracted. Hundred grams of arils were completely immersed in different solutions for 10 minutes and air dried.

T_1 - 0.1per cent KMS and 0.1per cent ascorbic acid

T_2 - 0.1per cent KMS and 0.1per cent citric acid

T_3 - 0.1per cent sodium benzoate and 0.1per cent ascorbic acid

T_4 - 0.1per cent sodium benzoate and 0.1per cent citric acid

T_5 - Calcium chloride (1%)

T_6 - Calcium ascorbate (1%)

T_7 - Sodium acid sulphate (3%)

T_8 - Control (without any treatment).

The treated arils were weighed, kept in aluminum foil tray, covered with its own lid and stored under ambient conditions. Physiological parameters such as physiological loss in weight, per cent leakage and chemical parameters like acidity (Ranganna,1991), TSS, vitamin C, carotenoids (Saini *et al.*, 2001) and total phenol (Sadasivam and Manikam, 1992) of treated arils were recorded continuously for a period of five days and average worked out. Based on superior parameters, the top four pre-treatments were selected for further microbial analysis, and based on efficiency in controlling the microbial populations, the best pre- storage treatment was selected for the next step.

Development of packaging system :

After surface sanitization and pre-storage treatment using the best chemicals selected in the part a and b of the experiments, respectively, minimally processed pomegranate arils were prepared, 100g. weighed and packed in following different packaging materials.

T_1- unventilated PE cover (150 gauge and 0.18 m^2 area)

T_2- unventilated PP cover (100 gauge and 0.18 m^2 area)

T_3- Ventilated PE cover (150 gauge and 0.18 m^2 area) with six pin holes on both sides.

T_4- Ventilated PP cover (100 gauge and 0.18 m^2 area) with six pin holes on both sides.

T_5- Aluminium tray with cling film (Klin wrap 300 mm) tightly wrapped around tray.

T_6- polystyrene tray with cling film (Klin wrap 300 mm) tightly wrapped around tray.

T_7- T_1+ $KMnO_4$ sachet

T_8- T_2+ $KMnO_4$ sachet

T_9- T_3+ $KMnO_4$ sachet

T_{10}- T_4+ $KMnO_4$ sachet

T_{11}- T_5+ $KMnO_4$ sachet

T_{12}- T_6+ $KMnO_4$ sachet

T_{13}- control (open storage in paper plate).

In treatments from T_7 to T_{12}, cloth sachet of 15cm^2 containing 1 per cent $KMnO_4$ was kept in packages for absorption of ethylene gas from inside the package. All packaged samples including control were stored under refrigerated condition maintained at 10-12°C. Physiological and chemical parameters were recorded as in part b. continuously for a period of five days and average worked out. Based on these different parameters the top ranking four packaging materials were selected and arils packaged in those selected packages were subjected to microbial analysis by serial dilution spread plate technique. Based on the efficiency in maintaining the microbial populations to the lowest level, the best packaging material was selected.

Acceptability of the standardized protocol :

Minimally processed pomegranate arils were prepared as per the developed protocol *viz.*, surface sanitization, treatment with pre-storage treatments followed by packaging and acceptability of the prepared arils were evaluated by conducting sensory evaluation by a 10 member semi trained panel using a nine-point hedonic scale.

Observations on pre-storage treatments and packaging were analyzed statistically in a Completely Randomized Design and significance was tested using analysis of variance technique (Gomez and Gomez, 1984).

EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Surface sanitization :

Fungal count on fruit surface was very negligible in all the treatments after sanitization. Surface sanitization with 120 ppm sodium hypochlorite resulted in least (1.39cfu × 10^3) bacterial population, which was on par with fruits treated with all concentrations of sodium hypochlorite solution. (Table 1). Uniform effectiveness may be due to the thick and smooth nature of the outer covering, hindering the action of sanitizer on it. Fruits washed in tap water had highest bacterial population (14.55cfu × 10^3). Chlorine compounds are usually used at levels of 50-200 ppm free chlorine and with typical contact times of less than 5 min (Francis and O'Beirne, 2002). Sodium hypochlorite is the source of chlorine commonly used in small-scale operations. For selection of any treatment, efficiency and economics are the two factors to be considered. If lower concentrations are equally effective as higher concentration, use of higher concentration can be avoided considering the safety and economics. As all the concentrations were equally effective, 30 ppm sodium hypochlorite solution was selected as best sanitizing agent for pomegranate. The effectiveness of sodium hypochlorite in the cleaning and disinfection processes depends on the concentration of available chlorine and the pH of the solution. Hypochlorous acid (HOCl) is a weak acid and dissociates to hypochlorite ion (-OCl) and proton (H+) depending on the solution pH. It is generally believed that HOCl is the active compound with germicidal action, whereas the concentration of -OCl is a key factor determining the cleaning efficiency (Fukuzaki, 2006). Fungal count was very negligible in all the treatments.

Evaluation of different pre-storage treatments :

Dipping fruit pieces into aqueous solutions containing

Table 1 : Bacterial population on surface of pomegranate fruit as influenced surface sanitization

Treatments	Bacterial population(cfu × 10^3)
40 °C water	13.46
30ppm sodium hypochlorite	11.33
60ppm sodium hypochlorite	2.27
90ppm sodium hypochlorite	8.00
120ppm sodium hypochlorite	1.39
Control (Washing with tap water)	14.55
C.D. (P=0.05)	10.76

Table 2 : Effect of pre-storage treatments on physiological and chemical parameters of pomegranate arils

Treatments	Physiological loss in weight (%)	Percentage leakage (%)	Acidity (%)	TSS (°B)	Vit. C (mg/100g)	Carotenoids (mg/100g)	Total phenol (mg/100g)
0.1% (KMS + Ascorbic acid)	2.66	61.50	0.055	14.64	42.13	0.007	192.20
0.1% (KMS + Citric acid)	2.26	70.92	0.050	14.76	42.80	0.003	209.40
0.1% (Sodium benzoate + ascorbic acid)	2.32	61.78	0.060	15.04	44.60	0.003	215.20
0.1% (Sodium benzoate + citric acid)	1.96	64.61	0.051	15.17	40.30	0.005	201.02
Calcium chloride (1%)	1.60	61.09	0.050	15.90	43.27	0.004	185.82
Calcium ascorbate (1%)	2.93	62.44	0.050	15.60	46.25	0.003	187.58
Sodium acid sulphate (3%)	6.74	89.35	0.066	16.09	40.19	0.004	185.50
Control (Without any treatment)	5.03	66.89	0.050	15.81	40.13	0.002	182.09
C.D. (P=0.05)	1.96	6.03	0.010	0.57	1.61	0.0003	15.69

antimicrobial agents, antioxidants or calcium salts is widely practiced to improve quality of any minimally processed fruit. After surface sanitization using 30 ppm sodium hypochlorite, rind of pomegranate fruits was removed, arils extracted and treated with different pre-storage chemicals.

Pre-storage treatments had influenced the physiological and chemical parameters of minimally processed pomegranate (Table 2). Physiological loss in weight (1.60) and per cent leakage (61.09) were least for the pomegranate arils treated with 1 per cent calcium chloride, hence were firm and succulent, which are the quality parameters of any fresh cut fruit. Chardonnet *et al.* (2003) reported calcium chloride as a widely used preservative and firming agent for fresh-cut commodities. Luna-Guzman and Barrett (2000) found that application of calcium chloride to cantaloupe melons makes the fruit firmer; the higher the concentration of calcium chloride used, the firmer the fruit.

Any fresh cut fruit should have low acidity and high total soluble solids for better palatability. Least acidity value (0.05) was expressed by all pomegranate arils except those treated with 3per cent sodium acid sulphate (0.066). Highest Total Soluble Solids was for the pomegranate arils treated with 1per cent calcium chloride (16.09) which was at par with the untreated arils (15.81)

Table 3 : Microbial count on pomegranate arils treated with selected pre-storage treatments and packaging materials

	Bacterial count (x10^3)	Fungal count (x10^3)
Pre-storage treatments		
0.1% KMS + ascorbic acid	14.33 (3.56)	0.50 (0.94)
0.1% sodium benzoate + citric acid	63.00 (6.03)	2.16 (1.53)
Calcium chloride (1%)	11.83 (3. 06)	1.00 (1.16)
Calcium ascorbate (1%)	3.17 (1.65)	0.83 (1.09)
C.D. (P=0.05)	2.73	0.36
Packaging materials		
Aluminium tray	5.50 (2.35)	0.50 (0.94)
Polystyrene tray	6.833 (2.59)	0.667 (0.99)
Aluminium tray+ $KMnO_4$ sachet	7.500 (2.75)	0.333 (0.88)
Polystyrene tray+ $KMnO_4$ sachet	5.667 (2.39)	0.667 (1.03)
C.D. (P=0.05)	NS	NS

Values in parenthesis show square root transformed values; NS = Non-significant

Table 4 : Effect of packaging materials on physiological and chemical parameters of minimally processed pomegranate arils

Treatments	Physiological loss in weight (%)	Percentage leakage	Acidity (%)	TSS (°B)	Vit. C (mg/100g)	Carotenoids (mg/100g)	Total phenol (mg/100g)
Unventilated polyethylene	5.47	20.32	0.050	14.92	46.36	0.030	219.50
Unventilated polypropylene	5.59	22.22	0.059	14.65	45.22	0.005	203.50
Microventilated polyethylene	5.61	23.02	0.050	15.57	48.65	0.005	232.83
Microventilated polypropylene	5.99	25.30	0.050	15.33	45.19	0.005	225.33
Aluminium tray	3.89	19.29	0.044	15.47	48.09	0.044	185.33
Polystyrene tray	5.18	19.56	0.044	15.43	47.90	0.008	187.50
Un ventilated PE + $KMnO_4$ sachet	7.45	23.75	0.059	14.95	42.33	0.003	205.03
Unventilated PP + $KMnO_4$ sachet	7.38	25.67	0.051	14.83	42.59	0.003	190.30
Microventilated PE + $KMnO_4$ sachet	7.30	25.88	0.066	15.40	43.29	0.024	217.00
Microventilated PP + $KMnO_4$ sachet	7.87	25.65	0.058	15.35	38.89	0.005	181.17
Aluminium tray + $KMnO_4$ sachet	4.96	19.19	0.043	15.27	44.33	0.038	187.33
Polystyrene tray + $KMnO_4$ sachet	4.65	18.29	0.044	15.20	43.30	0.045	181.83
Control	12.85	26.82	0.059	15.43	28.97	0.012	195.50
C.D. (P=0.05)	1.12	2.19	0.0138	0.76	2.43	0.0071	16.56

and arils treated with 1per cent calcium ascorbate (15.60). Highest TSS noticed in untreated arils might have been reduced by washing with other pretreatment chemicals.

Any processed product including minimally processed ones should retain the nutritional quality even after processing. Minimally processed pomegranate arils treated with 1per cent calcium ascorbate had highest vitamin C content (46.25) closely followed by sample treated with sodium benzoate+ ascorbic acid (44.60) and calcium chloride (43.27). Highest total carotenoid content was for the pomegranate arils treated with KMS + ascorbic acid (0.007).

Visual quality loss is the main factor limiting the shelf-life of minimally processed fruit produce. Hence, it should have low phenol content to avoid enzymatic browning. Total phenol was maximum for the untreated pomegranate arils (217.58). Arils treated with calcium chloride had least (182.09) phenol which was at par with arils treated with calcium ascorbate (185.50) and KMS + ascorbic acid (192.2). Calcium chloride has been one of the most frequently used salts of calcium and other calcium salts such as calcium lactate, calcium propionate or calcium ascorbate have been investigated as alternative sources of calcium (Dong *et al.*, 2000; Alandes *et al.*, 2006 and Quiles *et al.*, 2007).

Fan *et al.* (2009) identified sodium acid sulphate as an effective anti browning agent inhibiting microbial growth in apple slices. But in the present study arils treated with 3 per cent Sodium Acid Sulphate had highest PLW (6.74%) and per centage leakage (89.35), resulting in poor textural quality. It had poor colour too due to high (205.82) phenol content. Treatment with Sodium Acid Sulphate imparted a highly acidic taste to product and had lowest vitamin C content, hence not selected.

When the effect of all pre treatments were compared based on all physiological and chemical quality parameters, calcium chloride treated pomegranate arils recorded least PLW, percentage leakage, acidity, phenol and highest TSS. Calcium ascorbate treated arils had all these characters along with highest vitamin C. Calcium treatments can maintain or improve tissue firmness and crispness of minimally processed fruits. Calcium chloride has been one of the most frequently used salts of calcium and other calcium salts such as calcium lactate, calcium propionate or calcium ascorbate have been identified as alternative sources of calcium (Quiles *et al.*, 2007). Treatment combination of several chemicals was more effective than those applied individually and here combination of preservative and acidulant as a pre-storage treatment also had superior performance in maintaining the freshness and quality of minimally fruits. Pre-storage treatment with 0.1per cent KMS + ascorbic acid and 0.1per cent sodium benzoate + citric acid were effective for pomegranate arils. Potassium metabisulphite or sodium benzoate in combination with ascorbic/ citric acid was found to retain visual quality, enhance shelf-life and reduce microbial load during storage in fresh cut pineapple (Sheela, 2007). Sulphur dioxide in potassium metabisulphite has preservative action against bacteria and moulds and inhibits enzymes. It acts as an antioxidant; is a bleaching agent and is reported to be more effective when used along with acidulants like citric acid or ascorbic acid, rather than used alone (Varghese, 2006). The antimicrobial action of organic acids is due to pH reduction, disruption of membrane transport and permeability, anion accumulation as well as reduction in internal cellular pH. Similar result was obtained by Wang and Buta (2000). The antimicrobial action of organic acids is due to pH reduction, disruption of membrane transport and permeability, anion accumulation as well as reduction in internal cellular pH. Accordingly calcium chloride (1%), 0.1 per cent KMS + ascorbic acid, 0.1per cent sodium benzoate + citric acid and calcium ascorbate (1%), with superior quality parameters were selected for further microbial analysis.

Enumeration of total microbial load :

When microbial count on the arils treated with selected pre treatments was analysed, bacterial and fungal population were same for all the samples except those treated with 0.1 per cent sodium benzoate + 0.1per cent citric acid (Table 3). Bacterial (63 cfu × 10^3) and fungal (2.16 cfu × 10^3) count were maximum on arils treated with 0.1 per cent sodium benzoate+ 0.1per cent citric acid. As calcium ascorbate was very costly, it was not considered while selection. When physical quality parameters were also considered 1per cent calcium chloride had superior quality parameters compared to 0.1 per cent KMS + 0.1 per cent ascorbic acid and hence, selected as the best pre-treatment chemical for minimal processing of pomegranate arils. Calcium is reported to maintain firmness by cross-linking with cell wall and middle lamella pectin and it helps to maintain the fruit cell wall integrity by interacting with pectin to form calcium pectate.

Evaluation of packaging systems :

Minimally processed products must have a fresh appearance, visually acceptable, be of consistent quality throughout storage period in the package and be reasonably free of defects. Packaging materials have significant effect in reducing microbial population of fresh cut products (Varghese, 2006). Packaged cut materials are subjected to many stresses including deterioration reactions of wounded or senescing tissues, decay caused by growth of micro-organisms, water loss from the tissue, increase in respiration and ethylene production. These injuries induce severe damage and stress, with considerable reduction in shelf-life of commodity.

When different packaging materials were compared, trays showed superior quality parameters compared to covers. Retention of nutritional quality parameters like increased carotenoid and vitamin C was noticed in arils packed in these containers. Pomegranate arils kept in aluminium tray had lowest physiological loss in weight(3.89), per cent leakage (19.29), acidity (0.044) and phenol (185.33) along with increased vitamin C (48.09), TSS (15.47) and carotenoid (0.044), indicating their superiority. Pomegranate arils kept in polystyrene tray also had lowest per cent leakage(19.56), acidity (0.044) and phenol (185.33) along with increased vitamin C (47.9) and TSS (15.43). Addition of an ethylene absorbent also gave superior quality attributes to minimally processed fruits. Addition of $KMnO_4$, an ethylene absorbent, in aluminium and polystyrene trays could reduce acidity of pomegranate arils. When $KMnO_4$ sachet was kept along with arils in aluminium or polystyrene tray, they showed lowest physiological loss in weight, per cent leakage, acidity and phenol along with increased carotenoid. $KMnO_4$ removes ethylene from the system. Removal of ethylene from the storage environment of minimally processed fruits and vegetables can retard tissue softening (Abe and Watada, 1991).

Unventillated or microventillated packages, with or without an ethylene absorbent, were not always good in maintaining physiological quality parameters of minimally processed arils. Microperforated films having high gas transmission rates for oxygen and carbon dioxide can be used to extend shelf-life of highly respiring produce(Rai and Paul, 2007). All unpacked fruit pieces had highest physiological loss in weight. The fresh cut fruits are highly respiring in nature; hence, the films selected should have high gas transmission rates for oxygen and carbon dioxide. In the present study, unventilated or microventillated polyethylene or polypropylene with or without $KMnO_4$ sachet were found inferior to aluminium or polystyrene trays as it reduced the nutrient content of arils.

Considering all physical, chemical and physiological parameters, the top ranking four packaging materials *viz.*, aluminium tray and polystyrene tray with and without $KMnO_4$ sachet were selected for further microbial analysis.

Enumeration of total microbial load :

When microbial population was analyzed, both bacterial and fungal population were similar in pomegranate arils kept in all the selected packaging materials (Table 3). Addition of ethylene absorbent in the experiment could not make any added advantage on the microbial control. Instead, increased water droplets produced due to condensation favored spoilage of minimally processed produce, hence inclusion of a desiccant in the package, for water absorption would have been better than addition of an ethylene absorbent. Considering all chemical and physiological parameters, aluminium tray covered with cling film was selected as the best packaging material. Packaging of vegetables in trays and wrapping with cling film was also recommended by Varghese (2006) for enhanced keeping quality. They are the popular packaging materials for cut fruits, when presented as catering or snack trays. A view of the produce is also made possible in such materials.

Acceptability of the standardized protocol :

The quality of any food product could be defined by different ways from a widely manner to a more detailed one. One of the most usual meanings is defining the quality as "in conformity with consumer's requirements and acceptance is determined by their sensory attributes (Aumatell, 2009).

Minimally processed pomegranate arils prepared as per the standardised protocol were acceptable to the sensory panel even by the end of 5^{th} day. It was not having any visual symptom of microbial attack too. Minimally processed arils had the highest score of 9.0 on the day of preparation and it showed a decreasing trend and the score was 8.5 on 5th day of preparation. The microbiological, sensory and nutritional shelf-life of minimally processed vegetables or fruits should be at least 4-7 days (Ahvenainen, 2000), which was satisfied in the present study.

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