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DEVELOPMENT OF CALCIUM ENRICHED OSMO-DEHYDRATED APPLE SLICES BY IMPREGNATION AT ATMOSPHERIC PRESSURE

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ABSTRACT

The objective of this study was to develop calcium enriched osmo-dehydrated apple slices by impregnation at atmospheric pressure. Osmotic dehydration (OD) was carried out using 50°Bx sucrose solutions with 4% and 8% calcium lactate (CL) at atmospheric pressure for 16 hours followed by dehydration. It was found that, the calcium incorporated in 50 gm of osmo-dehydrated apple slices meets up to 27% of the, Recommended Dietary Allowance (RDA) of calcium for adults in India. Moisture content of all the samples were much below the maximum limit of moisture content (20% m/m). Non-enzymatic browning was significantly ($p<0.05$) lower in slices which were treated with calcium. Microbiological quality was better in sample treated with 8% calcium lactate. The sensory panel did not observe any significant differences in appearance, flavor, sweetness, texture and overall acceptability in any of the sample. Thus, it can be concluded that a healthy and nutritious ready to eat calcium enriched apple slices were developed by applying simple and inexpensive atmospheric impregnation method without affecting sensory characteristics.

Keywords: *Calcium Lactate, Osmotic dehydration, Atmospheric Pressure, Apple.*

I. INTRODUCTION

Calcium is the most abundant mineral present in the body and is required for growth and maintenance of human body. Deficiency of calcium occurs in large segments of the populations of both developed and developing countries, and has been linked to several chronic diseases, including colon cancer, hypertension osteoporosis, and osteomalacia (Goldberg, 1994). If milk and dairy products are not a regular part of the diet, there are few excellent dietary sources of calcium. Dietary calcium intake is inadequate in both rural and urban population compared to the recommended daily allowances (RDA) for our country (Harinarayan et al, 2004). The difference between the intake of calcium and recommendations has led manufacturers to market an increasing number and variety of calcium fortified or calcium enriched products (Singh et al, 2006). These functional products include those designed with added physiologically-active food components (PAC) (Clare & Hasler, 1998; IOM/NAS, 1994).

In fruit tissues, the PAC can be incorporated through vacuum impregnation (VI) method. However, impregnating calcium into fruit tissues using vacuum impregnation is an expensive technology. Therefore, there is a need for inexpensive method to impregnate calcium in fruits. Impregnating calcium at atmospheric pressure (AI) has recently received increased attention as potential processes for the design of new enriched fruit and vegetable products. In this method, solutes can be incorporated through impregnation, to increase the product nutritional characteristics (Mujica-Paz et al, 2003).

Osmotic dehydration allows the introduction of solute in tissue of fruits which favor sensory and nutritional characteristics products (Fito et al, 2001). During the osmotic process, there are two major simultaneous counter current flows: flow of water from the food into the osmotic solution and flow of solutes from the solution into the food (Dixon & Jen, 1977), thus osmotic solution can be used as an impregnating medium. Apple has considerably high porosity filled with gas which makes this fruit suited to calcium impregnation. Therefore, apple tissue could be used as a good matrix for calcium fortification (Anino, 2006). Dehydrated enriched apple slices when converted to powder form could also be used as an ingredient to be included in complex foods such as bakery products, ice cream, confectionaries, dairy and cereal products thus increasing the calcium contents of the products. The objective of this study was to develop calcium enriched apple slices by use of osmotic solution at atmospheric pressure (AI). The physicochemical, microbiological and sensory characteristics of the osmo-dehydrated apple slices were evaluated.

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II. MATERIALS AND METHODS

II (a) Raw Materials

The Red Delicious variety of apples (10°Bx and pH 4.4-4.8) free from any blemishes were procured from Safal Outlet (Bengali Market), Delhi and maintained at 4°C until use. The osmotic and impregnating solutions were prepared using commercial sucrose (amorphous refined sugar) purchased from a local market; food grade calcium lactate in powder form obtained from pioneer Inorganics, Tilak Bazar, Delhi and distilled water.

II (b) Preparation of Osmotic and Impregnating solutions

In osmotic treatment, a 50°Bx sucrose solution was used as the osmotic solution. In order to incorporate calcium into the porous structure of the apple slices, calcium lactate was added to the osmotic solutions. This salt was chosen as the calcium source because of its relatively high bioavailability and solubility at room temperature, in addition to the neutral taste that it imparts to the fruit. Atmospheric Impregnating solutions were prepared by using 4% and 8% of calcium lactate in the 50°Bx osmotic solution.

II (c) Experimental Methodology

Apples were washed, cored and sliced into 2 mm thickness using apple slicer without peeling and then dipped immediately in brine (2%) solution to prevent browning, till all the slices were cut. The cut slices were then blanched and then kept into vessels containing the osmotic and impregnating solutions at 40°C, in a 1:10 mass ratio of fruit to solution (w/w). The treatment conditions are given in table 1. The vessels were then covered with plastic film and placed in an incubator at 40°C. The slices were taken out after 16 hours of osmotic dehydration. After this process, the slices were quickly rinsed with distilled water, then weighed and dehydrated (70°C for 8 hours) in cabinet drier. The developed osmo-dehydrated and dried apple slices were immediately packed in low density polypropylene bags and kept for physicochemical, microbiological and sensory analysis.

Table 1: Treatments given to apple slices for osmotic dehydration

Sample	Treatment [Sucrose (°Bx) + Calcium lactate (%)]
Control	50 + 0
A	50 + 4
B	50 + 8

Similarly, the apple slices treated in these solutions were Control, A and B, respectively.

II (d) Physicochemical Analysis

Moisture, total ash, calcium content, pH, titratable acidity, total soluble solids (TSS), total sugars and non-enzymatic browning (NEB) were determined as reported by Ranganna (1986). Weight loss (WL)/Moisture Loss (ML) and Solid Gain (SG) of apple slices were calculated using Equations 1 & 2, respectively.

$$WL/ML\ \% = \frac{(w_i - w_f)}{w_i} \times 100 \quad (1)$$

Where: w_i is weight/moisture initial and w_f is weight/ moisture final.

$$SG\ \% = \left(\frac{{}^{o}Bx_f - {}^{o}Bx_i}{{}^{o}Bx_i}\right) \times 100 \quad (2)$$

Where: ${}^{o}Bx_i$ represents initial °Bx & ${}^{o}Bx_f$ represents final °Bx (Zapata et al, 2011).

II (e) Microbial safety

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Microbial assessment was done by colony forming units (cfu) with pour plate techniques by using nutrient agar (NA) and potato dextrose agar as media for bacteria and fungi, respectively (IS: 5402-2002; IS: 5403-1999) and Violet Red Bile Agar for coliform counts (IS: 5401-2002).

II (f) Sensory analysis

Prior to sensory analysis the panellist were given a training to familiarize them with the desirable and undesirable characteristics of the product. Sensory evaluation was done by 25 semi-trained panellists within the age group of 20 to 25 years. Apple slices were evaluated for sensory appearance, color, firmness, taste and overall acceptability on a 5-point hedonic scale where 5 = like very much; 1 = dislike very much.

II (g) Statistical Analysis

All the results in triplicate were analyzed using ANOVA by using SPSS version 22.

III. RESULTS AND DISCUSSION

III (a) Physicochemical Characteristics

Calcium

Table 2 shows the calcium content of fresh and osmo-dehydrated apple slices treated with and without calcium lactate. It was observed that calcium content was significantly ($p<0.01$) lost after osmotic dehydration (OD) in control (50°Bx) as compared with fresh apple. This can be explained by diffusion of soluble solids from fruit to osmotic solution. Similar trend was observed by Peiro et al, 2006, Stojanovic and Silva, 2007. Calcium content in apple slices treated with 4% Calcium Lactate (CL) was 212 mg/100gm and apple slices treated with 8%CL was 319 mg/100gm thus meeting 17.6% and 27%, respectively, of the Recommended Dietary Allowance (RDA) (ICMR, 2009) of calcium for adults in a 50 gm sample (usual serving size for solid food) (Allen, 2006). The gain in calcium significantly ($p<0.01$) increased with increase in the concentration of calcium lactate in the osmotic solution. According to U.S. Food and Drug Administration (FDA), food product can be claimed as "Excellent Source" of particular nutrient if it contains 20% or more of the Daily Value (DV) per Reference Amount Customarily Consumed (RACC) and can be claimed as "Good Source," if it contains 10%-19% of the DV per RACC. Accordingly, Sample A can be claimed as “Good source” of calcium and “sample B” can be claimed as “Excellent source” of calcium.

Weight Loss (WL)/Moisture Loss (ML)

Table 3 demonstrates the WL/ML after OD in apple slices. Both WL and ML were affected by different concentrations of calcium lactate, at the same sucrose concentration. Addition of 4% and 8% calcium lactate significantly ($p<0.01$) increased the WL and ML from apple slices. Similar results have been

Table 2: Calcium content of apple slices (Fresh, Control, A and B)

Sample*	Calcium content (dry basis) mg/100g	% of RDA per 50 gm
Fresh apple	65.845 ± 1.055^{a}	5
Control (50°Bx)	8.25 ± 0.15^{b}	0.65
A (50°Bx + 4%CL)	212.37 ± 0.32^{c}	17.6
B (50°Bx + 8%CL)	325.7 ± 0.27^{d}	27

*Results are expressed as the Means ± S.E. for triplicates

^{a,b,c,d}mean scores, bearing different superscripts in a column differ significantly ($p<0.05$)

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reported by Silva et al, 2014, they observed that the addition of calcium to the osmotic solution reduced the moisture content of the product. There was also significant ($p<0.01$) WL and ML in between samples A and B. Highest WL/ML loss occurred in Sample B. This could be due to higher concentration of calcium lactate (8%) in the osmotic solution, which results in more loss of water from the fruit to the solution therefore resulting highest WL/ML (Silva et al, 2014).

Solid Gain (SG)

Table 3shows the solid gain in apple slices treated with different concentrations of calcium lactate. The apple slices treated with 50°Bx (control) caused significantly ($p<0.05$) greater sucrose incorporation when compared with samples treated with different concentrations of CL. It could be because of the presence of CL in the osmotic solution which tends to restrict the gain in sucrose from osmotic solution to apple slices. Similarly Silva et al, 2014 also studied the reduction in sucrose impregnation rate after addition of CL in osmotic solution. The addition of CL in osmotic solution significantly ($p<0.05$) reduced the sucrose gain in samples, irrespective of the concentration of calcium lactate. Mavroudis et al, (2012) observed that the solute gain in apples decreased with the addition of 0.6% calcium lactate to the solution, and attributed the result to a reduction in cell wall porosity. The limited transfer of sucrose into apple tissue could be attributed to the pectin and enzymes present in this fruit. The hydrolysis of pectin methyl esters by pectin-methyl esterase (PME), an important enzyme in apple (Silva et al, 2011a and Silva et al, 2011b), generates carboxyl groups that can interact with calcium (Guillemin et al, 2008), promoting cross-linking of the pectin polymers that can reinforce the cell walls (Anino et al, 2006). Since cuts and injuries to the tissue provoke the release of enzymes, calcium pectate could be formed around the cut surfaces, which in turn would act as a partial barrier to the diffusion of larger molecules such as sucrose into the tissue (Barrera et al, 2009; Silva et al, 2013). Addition of 1% calcium lactate to the osmotic solution decreased the constant rate of gain in soluble solids, but resulted in a final product with an increased mineral content (Barrera, 2009).

Ash

Table 3 shows the ash content of treated samples (control, A and B). There was significant difference ($p<0.01$) in ash content between all treated samples. Ash content was significantly ($p<0.01$) higher in sample B (50°Bx + 8% CL) than in sample A (50°Bx + 4% CL) and lowest in control (50°Bx), due to higher concentration of CL in osmotic solution of sample B, then in sample A resulting more impregnation of CL in sample B when compared with sample A (Silva et al, 2014).

pH and Titratable acidity

Table 3 shows the pH and titratable acidity of osmo-dehydrated apple slices. There was no significant difference in pH and titratable acidity between samples treated with and without calcium. Since, all the samples were soaked in same concentration of sucrose and for same time, thus, they would have lost almost similar amount of acids into the osmotic solution.

Total Soluble Solids (TSS) and Total Sugars

Table 3 shows TSS and total sugars of osmo-dehydrated apple slices which shows there was significant difference ($p<0.05$) between control and samples A and B. Control sample has higher values of TSS and total sugars as compared with samples A and B due to more diffusion of sucrose in control sample then in sample A and B. Cell wall porosity of apple gets reduced due to impregnation of calcium resulting sucrose inhibition. Calcium acts as a partial barrier to the diffusion sucrose into the tissue (Barrera et al, 2009; Silva et al, 2013). No significant difference in TSS and total sugars was between samples of A and B.

Moisture Content

Table 3 shows moisture content of osmo-dehydrated apple slices. There was no significant differences in the moisture content of control, A and B samples. According to Food Safety and Standard Regulation (2010), moisture content in dehydrated fruits should not be more than 20% m/m. Moisture content in Control, A and B samples were far below the recommended standard value therefore indicating that drying conditions were efficient.

Non-Enzymatic Browning

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Table 3 shows non-enzymatic browning of osmo-dehydrated apple slices. Non enzymatic browning was significantly ($p<0.05$) higher in control sample when compared with samples A and B. Baloch and khan, 1997 and Simon et al, 1955 have reported that calcium may block the amino group, thus restricting it from entering into the browning reaction. They also believed that calcium is capable of forming chelating compounds with organic substances having an alpha amino carboxylic acid structure. Therefore, it would be reasonable to expect that calcium treatment was applicable to control non enzymatic browning in samples A and B which were treated with CL.

III (b) Microbial counts

Table 4 shows total plate count, *coliforms* and yeast and mould count apple slices. Yeast and mould count was not detectable in any of the samples (control, A and B), indicating all the samples were in a good condition. *Coliform* count was also not detectable in any of the three samples which indicated that the

Table 3: Physicochemical characteristics of apple slices (Fresh, Control, A and B)

Parameters*	**Control (50°Bx)**	**A (50°Bx + 4% CL)**	**B (50°Bx + 8% CL)**
Moisture Content (%)	2.16 ± 0.035^{a}	2.15 ± 0.05^{a}	2.1 ± 0.1^{a}
Weight loss (%)	28.7 ± 0.2^{a}	37.8 ± 0.4^{b}	44.75 ± 0.15^{c}
Moisture loss (%)	20.35 ± 0.04^{a}	25.36 ± 0.07^{b}	31.69 ± 0.05^{c}
Solid gain (%)	3.32 ± 0.09^{a}	3.22 ± 0.08^{b}	3.22 ± 0.013^{b}
Non-Enzymatic Browning (NEB)	0.00415 ± 0.00015^{a}	0.00195 ± 0.00005^{b}	0.0015 ± 0.00005^{c}
Ash (%)	0.61 ± 0.01^{a}	1.60 ± 0.015^{b}	2.65 ± 0.05^{c}
pH	5.25 ± 0.05^{a}	5.35 ± 0.05^{a}	5.3 ± 0.5^{a}
Titratable Acidity (%)	0.23 ± 0.0005^{a}	0.21 ± 0.001^{a}	0.213 ± 0.0005^{a}
Total Soluble Solids (°Bx)	43.25 ± 0.25^{a}	40.25 ± 0.25^{b}	40 ± 0^{b}
Total Sugars (%)	77.1 ± 0.1^{a}	65.25 ± 0.15^{b}	64.35 ± 0.35^{b}

*Results are expressed as the Means ± S.E. for triplicates
^{a,b,c}mean scores, bearin-g different superscripts in a row differ significantly ($p<0.01$)

hygienic practices and the sanitization process applied were effective. TPC growth was lower in samples A and B (treated with CL) as compared to control. The treatment with calcium lactate seems to imply an improvement of microbiological fruit quality. This can be related to the reduction of the cellular metabolism caused by the increase in the intracellular ATP concentration due to the effect of calcium. Castello et al, 2009 studied the influence of osmotic dehydration on microbial stability of apple slices and they reported that calcium improved the shelf life of OD apple slices. Similar results were also obtained by other authors, who reported that calcium treatments extend the shelf life of apple slices (Abbott et al, 1989), of fresh-cut cantaloupe melon (Luna-Guzman and Barrett, 2000) and fresh-cut mango (Torres et al, 2008).
According to the microbiological criterion recommended for fruits and vegetables, the maximum limits for mesophilic aerobic bacteria and yeast and mould are 1×10^{4} cfu/g and 1×10^{2} cfu/g, respectively (Pascual and

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Calderon, 2000). The maximum limit for TPC according to Food Safety and Standard Regulations (2010) for dehydrated fruits is 4×10^4 cfu/g. All the three samples did not exceed recommended value after processed indicating that drying conditions were efficient. All the osmo-dehydrated apples were safe to eat.

III (c) Sensory characteristics

Table 5 shows the mean score obtained for all samples treated

Table 4: Microbial counts (cfu/g)) of total plate count (TPC), *coliforms*, yeasts and moulds (Y&M) of apple slices

Parameters	Sample		
	Control (50°Bx)	A (50°Bx + 4% CL)	B (50°Bx + 8% CL)
TPC (cfu/g)	8.2X10	5.45X10	3.63X10
Coliforms (cfu/g)	ND	ND	ND
Yeast & Mould (cfu/g)	ND	ND	ND

Where, Not Detectable (ND) means <1 cfu/g

with and without calcium lactate. The sensory panel observed no significant difference in appearance, flavour, sweetness, texture and overall acceptability between control, A and B sample. All the samples scored above the limit of saleability i.e. 3 on 5-point Hedonic scale as given by Rico et al, 2007. Thus, calcium lactate impregnation in apple slices can be carried out without significantly affecting sensory characteristics.

Table 5: Sensory characteristics of apple slices (Fresh, Control, A and B)

Parameters	Sample		
	Control(50°Bx)	A (50°Bx + 4%CL)	B (50°Bx + 8%CL)
Appearance	4.0±0.62	4.0±0.54	4.0±0.39
Flavor	4 ±0.55	4 ±0.38	4.03±0.12
Sweetness	3.9±0.63	4.1±0.70	4.0±0.66
Texture	4±0.75	4.0±0.56	4.0±0.66
Overall acceptability	3.9±0.54	4.1±0.47	4.1±0.45

*Results are expressed as the Means ± S.E. for triplicates

IV. CONCLUSION

It was concluded that impregnation of calcium in apple slices can be done successfully when combined with osmotic dehydration method. It increased the calcium content in apple slices. Calcium content in apple slices treated with 4% CL and 8% CL were meeting up to 17.6% and 27%, respectively, of the Recommended Dietary Allowance (RDA) of calcium for adults in a 50 gm sample. Therefore, healthy and nutritious ready-to-eat calcium enriched apple slices were developed with good sensory characteristics like appearance, flavour, sweetness, texture and overall acceptability, thus helping promote the consumption of calcium in human diets.

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