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EFFECT OF CALCIUM LACTATE ON THE SHELF LIFE OF OSMOTICALLY DEHYDRATED APPLE SLICES

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ABSTRACT

The effect of calcium lactate on osmotically dehydrated apple slices was monitored during storage. 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. Samples were periodically evaluated for physicochemical characteristics, microbiological quality and sensory acceptance, stored at 25 ± 2ºC for 120 days at an interval of 15 days. Moisture content of all the treated samples increased significantly ($p<0.05$) during storage. The calcium salt significantly ($p<0.05$) reduced the non-enzymatic browning in samples treated with calcium lactate during storage. Maximum microbial stability was observed in the samples treated with 8% calcium lactate during storage. The sensory panel did not observe any significant differences in appearance, flavor, sweetness, texture and overall acceptability between dried apple slices treated with or without calcium lactate during storage. Thus, it can be concluded that calcium lactate increased the physicochemical characteristics, microbiological quality without affecting sensory characteristics of apple slices up to 120 days of storage.

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

I. INTRODUCTION

Apple (*Malus domestica*) is one of the leading fruits which are being grown in temperate region of the world. Its beautiful appearance, crispy flesh, pleasant flavour and sweet taste attract the consumers (Ali et al, 2004). Apple consumption has increased in India. According to industry, demands for fresh apples are growing in both urban and rural India. The major factors fueling apple demand are increasing population, growing disposable incomes, improving lifestyle, health awareness, and India's tradition of vegetarianism (Linda et al, 2007).However, apples contains high amount of water thus, it perishes quickly. In order to achieve their stability, the water contained in them should be reduced to a certain minimum limit (Gyurova, 2014; Park, 2005). Osmotic Dehydration (OD), water removal process can be employed to obtain minimally processed food with a longer shelf life. As a pre-treatment to dehydration, OD can reduce the moisture content of a fruit by approximately 50%, can also reduce aroma losses and enzymatic browning and increase sensory acceptance (Ponting et al, 1996; Torreggiani & Bertolo, 2001; Pan et al, 2003; Lombard et al, 2008). However, OD leads to some structural changes in the product (Sormani et al, 1999; Mastrangelo et al, 2000; Pereira et al, 2007). This leads to loss of cell turgidity, deformation and/or cell wall rupture, splitting and degradation of the middle lamella, lysis of membrane, cellular collapse, plasmolysis and tissue shrinkage (Lewicki & Pawlak, 2005).The addition of calcium salts to osmotic solutions has been used to reduce the damage caused to the structure of the cell wall due to dehydration (Ferrari et al, 2010). In the case of non-processed whole or fresh-cut fruit and vegetables, calcium salts act as texture agents. The treatment of fresh fruit with calcium has a significant influence on its firmness; e.g., it delays unfavourable textural changes, improves tissue structure, decreases susceptibility to disease during storage, lengthens shelf-life and maintains the firmness and quality of fruit (Barrett et al, 1998; Chardonnet et al, 2003; Anino et al, 2006; González et al, 2008). Calcium ions feature in maintaining plant cell functioning, structure and stability through preserving cell wall integrity. Low methylated pectin molecules are cross-linked by calcium bridges, which have a significant influence on ''tightening" the cell walls and results in increased tissue firmness. Calcium bridges are formed between free carboxyl groups of pectin chains. Presence of calcium influences the middle lamella by delaying the separation of cells during ripening (Johnston et al, 2002).The use of calcium salts have been reported to have an antimicrobial effect in fruits and vegetable (Luna-Guzman &Barrett 2000; Martin-Diana et al, 2005). Pereira et al 2010, studied that calcium lactate inhibited microbial growth on guavas, with yeast and mold counts in the order of 10^2 cfu/g throughout storage and tissue structure preservation was also be attributed to calcium lactate use. Thus, the objective of this work was to verify the effect of calcium lactate on the shelf life of osmotically dehydrated apple slices, by evaluating physicochemical characteristics, microbiological quality and sensory acceptance of the fruits during 120 days storage.

II. MATERIALS & METHODS

a. Raw Materials

The Red Delicious variety of apples (10°Bx and pH 4.4-4.8) were procured from Safal Outlet (Bengali Market), Delhi and maintained at 4°C until use. The osmotic 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.

b. Preparation of Osmotic 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 salt was added to the osmotic solutions at 4% and 8% respectively. 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.

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 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 apple slices were immediately packed in low density polypropylene bags (LDPE) and kept for storage for 120 days at 25 ± 2ºC.Stored samples were analyzed for physicochemical, microbiological and sensory characteristics for 120 days at an interval of 15 days.

d. Physicochemical Analysis

Moisture content and non-enzymatic browning (NEB) were determined as reported by Ranganna (1986).

e. Microbiological Quality

Microbiological analyses were carried out during storage to determine the total plate count (TPC), yeast and mold count. The coliform group was also determined during storage to evaluate the hygienic conditions of the raw material, processing and storage conditions. Microbial assessment was done by colony forming units (cfu) of apple slices 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).

f. Sensory analysis

Sensory acceptance tests during storage were carried out. Prior to sensory analysis the panelist were given a training to familiarize them with the desirable and undesirable characteristics of the product. Sensory evaluation was done by 25 semi-trained panelists 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.

g. Statistical Analysis

All the results in triplicate were analyzed using ANOVA and t-test by using SPSS version (22).

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III. RESULTS & DISCUSSIONS

a. Physicochemical Characteristics

Moisture content

Table 1 shows the changes in moisture content in osmotically dehydrated apple slices during storage. There was significant (p<0.05) increase in moisture content in all osmotically dehydrated apple slices. There was some amount of moisture ingress in samples during storage and it increased as the duration of storage increased. The increase in moisture content was observed due to packaging material that may have caused migration of water into the pouch, as the samples were stored at ambient temperature. This hypothesis was verified by Manzano et al, (1997) that storage temperature affects the moisture content of fruits during storage. Similar results were also reported by Kumar et al, (2008), Rahman et al, (2010) and Mizanur et al, (2012).Control sample has significantly (p<0.05) higher sucrose in it, compared to samples A and B, and sucrose are hygroscopic in nature therefore, they might have absorbed moisture from the surroundings and thus it started gaining moisture early.

Non Enzymatic Browning (NEB): Table 2 shows the changes in non enzymatic browning in osmotically dehydrated apple slices during storage. Non enzymatic browning was significantly (p<0.05) higher in control sample when compared with samples A and B which were treated with calcium lactate on 0-day.

Table 1: Changes in moisture content* in all treated osmotically dehydrated apple slices during storage

No. of Days	Moisture content (%)		
	Control(50 °Bx)	**A(50°Bx + 4% CL)**	**B(50°Bx + 8% CL)**
0	2.16 ± 0.035	2.15 ± 0.05	2.1 ± 0.1
15th	2.2 ± 0.05	2.23 ± 0.05	2.14 ± 0.1
30th	2.34 ± 0.05	2.35 ±0.03	2.23 ± 0.1
45th	2.45 ± 0.05^{a}	2.4 ±0.05	2.36 ± 0.02
60th	2.675 ± 0.02^{b}	2.56 ± 0.05	2.4 ± 0.05
75th	2.75 ±0.05^{c}	2.63 ± 0.05	2.61 ± 0.05
90th	2.85 ± 0.05^{d}	2.78 ± 0.05^{a}	2.71 ± 0.005
105th	3.09 ± 0.01^{e}	2.9 ± 0.05^{b}	2.8 ± 0.05^{a}
120th	3.25 ± 0.05^{f}	3.4 ± 0.045^{c}	3.01 ± 0.015^{b}

*Results are expressed as the Means ± S.E. for triplicates
a,b,c,d,e,f mean scores, bearing different superscripts in a column differ significantly (p<0.05).

Baloch & 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

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reasonable to expect that calcium treatment was applicable to control non enzymatic browning in samples A and B which were treated with CL.

a. **Microbiological Quality**

Table 3 shows total plate count, *coliforms* and yeast and mould count of all osmotically dehydrated apple slices during storage. Yeast and mould count was not detectable in any of the samples till 120th day of storage, indicating drying and storage conditions were efficient. *Coliform* count was also not detectable in any of the three samples which indicated that the hygienic practices and the sanitization process applied were effective. The total plate count increased during storage in all the samples. 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.

Table 2: Changes in non enzymatic browning* in osmotically dehydrated apple slices treated with and without the addition of calcium lactate during storage

No. of Days	Non Enzymatic Browning		
	Control(50° Bx)	A(50°Bx + 4% CL)	B(50°Bx + 8% CL)
0	0.00415±0.00015^{a}	0.00195±0.00005^{a}	0.0015±0.0005^{a}
15th	0.0071±0.00009^{b}	0.00415±0.00015^{b}	0.0125±0.0025^{b}
30th	0.0088±0.0002^{c}	0.018±0.001^{c}	0.018±0.002^{c}
45th	0.012±0.002^{d}	0.042±0.002^{d}	0.0285±0.0015^{d}
60th	0.023±0.002^{e}	0.042±0.001^{e}	0.0315±0.0015^{e}
75th	0.0395±0.0005^{f}	0.044±0.002^{f}	0.036±0.001^{f}
90th	0.0435±0.0005^{g}	0.0475±0.0015^{g}	0.0395±0.0005^{g}
105th	0.0445±0.0005^{h}	0.05±0.001^{h}	0.0435±0.0015^{h}
120th	0.054±0.001^{i}	0.0585±0.0015^{i}	0.0465±0.0015^{i}

*Results are expressed as the Means ± S.E. for triplicates
a,b,c,d,e,f,g,h,i, mean scores with different superscripts within a column differ significantly ($p<0.05$)

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 & Barrett, 2000) and fresh-cut mango (Torres et al, 2008).

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a. Sensory characteristics

Table 4 shows the mean score obtained for all samples treated with and without calcium lactate, evaluated from 0 to 120th day at an interval of 15 days. The sensory panel observed no significant difference in appearance, flavour, sweetness, texture and overall acceptability between control, A and B sample during storage. 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 3: Microbial counts (cfu/g)) of TPC, coliforms, yeasts and moulds (Y&M) of osmotically dehydrated apple slices during storage

Parameters	**Treatments**	**No. of Days**								
		0	**15th**	**30th**	**45th**	**60th**	**75th**	**90th**	**105th**	**120th**
TPC (cfu/g)	**Control**	8.2×10	1.18×10^{2}	1.36×10^{2}	1.81×10^{2}	1.90×10^{2}	2.72×10^{2}	2.54×10^{2}	3.63×10^{2}	4.63×10^{2}
	A	5.45×10	7.27×10	8.18×10	1×10^{2}	1.09×10^{2}	1.18×10^{2}	1.54×10^{2}	1.72×10^{2}	1.90×10^{2}
	B	3.63×10	5.45×10	6.36×10	9.09×10	1.09×10^{2}	1.09×10^{2}	1.27×10^{2}	1.36×10^{2}	1.54×10^{2}
Coliforms (cfu/g)	**Control**	ND	ND	ND	ND	ND	ND	ND	ND	ND
	A	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yeast & Mould (cfu/g)	**Control**	ND	ND	ND	ND	ND	ND	ND	ND	ND
	A	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	ND	ND	ND	ND	ND	ND	ND	ND	ND

Where, Not Detectable (ND) means <1 cfu/g
Control (50°Bx), A (50°Bx + 4%CL), B (50°Bx + 8%CL)

Table 4: Sensory characteristics of osmotically dehydrated apple slices during storage

Days	**Appearance**			**Flavor**			**Sweetness**			**Texture**			**Overall acceptability**		
	Control	**A**	**B**	**Control**	**A**	**B**	**Control**	**A**	**B**	**Control**	**A**	**B**	**Control**	**A**	**B**
0	4.0±.62	4.0±.54	4.0±.39	4±.55	4±.38	4.03±.12	3.9±.63	4.1±.70	4.0±.66	4±.75	4.0±.56	4.0±.66	3.9±.54	4.1±.47	4.1±.45
15th	4.2±77	4.1±.79	4.3±.48	3.9±.81	4±.77	4.2±.41	4.2±.64	4.0±.66	4.3±.48	4.1±.74	4.2±.77	4.3±.61	4.0±.68	3.9±.66	4.4±.47
30th	4.0±.65	4.0±.65	3.9±.66	4.0±.53	4.2±.41	4.1±.60	3.8±.63	4.1±.45	3.9±.54	3.9±.59	4.0±.66	4.0±.32	4.2±.67	4.1±.35	4.1±.60

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45th	4.1±45	4.2±.36	4.1±.35	4.0±.37	3.9±.25	3.8±.35	4.0±.37	3.9±.25	3.8±.41	3.9±.76	3.9±.45	3.9±.50	4.1±.35	4.0±.25	3.8±.35
60th	4.1±.35	4.3±.48	4.0±.00	4.3±.48	4.2±.41	4.1±.25	4.4±.50	4.3±.48	4.2±.45	4.3±.45	4.2±.41	4.0±.25	4.2±.41	4.1±.35	4.1±.35
75th	4.3±.48	4.3±.48	4.3±.45	4.3±.45	4.1±.35	4.5±.51	4.3±.44	4.3±.45	4.2±.41	4.4±.50	4.3±.48	4.3±.48	4.2±.41	4.2±.45	4.2±.41
90th	4.1±.35	4.2±.41	4.2±.41	4.3±.45	4.3±.48	4.3±.45	4.3±.61	4.2±.41	4.4±.50	4.2±.56	4.3±.48	4.0±.45	4.3±.48	4.2±.41	4.0±.25
105th	4.3±45	4.3±.70	4.3±.45	3.9±.51	4.1±.25	4.1±.56	4.2±.59	3.9±45	4.0±.45	4.0±.37	4.2±.67	4.0±.45	4.0±.45	4.2±.41	4.0±.25
120th	4.2±.41	4.0±.65	4.1 ± 45	4.0±.37	4.2±.56	4.0±.53	3.9±.61	3.8±.51	4.0±.25	4.4±.63	4.2±.56	4.0±.59	4.0±.37	4.2±.41	4.0±.37

Where, Control (50°Bx), A (50°Bx + 4%CL), B (50°Bx + 8%CL)

IV. CONCLUSION

Calcium lactate improved the physicochemical characteristics of apple slices treated with calcium lactate. There was no significant differences in appearance, flavor, sweetness, texture and overall acceptability between apple slices treated with or without calcium lactate during storage. Samples treated with calcium lactate also scored above the limit of saleability i.e. 3 on 5-point Hedonic scale as given by Rico et al, 2007. Thus, it can be concluded that calcium lactate increased the physicochemical characteristics, microbiological quality without affecting sensory characteristics of apple slices up to 120 days.

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