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Proximate Composition and Preservation by Combined Methods of Chupandia (*Cyrtocarpa procera*) Pulp

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Abstract: The *Cyrtocarpa procera* pulp was analysed for proximate composition and the effects of combining techniques such as addition of Potassium Sorbate (KS), modification of water activity (a_w) and Heat treatment (H) on the microbiological shelf life of *C. procera* pulp were evaluated during 100 days of storage at 25°C. The experiment was laid out in a randomized 2^3 factorial with three replications. The pulps were periodically analyzed for UFC/g of yeast and moulds, pH, a_w and total soluble solids (°Brix)). The values (%) of proximate analyses were: moisture 83.62, protein 1.61, fat 0.30, ash 0.68, crude fiber 0.38 and carbohydrate 13.41. Metabolizable energy 62.78 kcal/100 g. The addition of 400 ppm KS ($p<0.0001$) and H (60-65°C for 3 min, $p<0.05$) can extend the stability of the pulp up to 100 days at ambient temperature (25°C) without deterioration in quality. The reliability of a model developed to predict the a_w 25°C as a function of pH and °Brix, was assessed through validation with the *C. procera* pulp results, the predictive performance of the model can be considered acceptable. Results indicate that combining heat treatment with addition of KS can be used to extend the shelf life of *C. procera* pulp kept at ambient temperature and that the low carbohydrate and gross energy values may qualify *C. procera* pulp as a good component in food formulation for the obese and diabetics.

Key words: *Cyrtocarpa procera*, combined methods, water activity, pH, total soluble solid, fruits

INTRODUCTION

Fresh fruits and vegetables have both economic and nutritional value. The National Cancer Institute encourages people to eat vegetables and fruits as part of a healthy diet to reduce risk of chronic diseases such as cancer, heart diseases and Alzheimer´s (NCI, 2001). The amount of fruits and vegetables required by an individual per day is at least 400 g (FAO/WHO, 2004). Growing and marketing of fresh fruit and vegetables are complicated by post-harvest losses in quantity and quality between harvest and consumption. Many edible indigenous fruits are nutritious, tasty and contribute to the food security of rural households. But there are very few indigenous fruit products available in the shops. Indigenous fruits are characterized by mass fruiting seasons that last only for a couple of months. This seasonality can cause supply/demand imbalances and a collapse in price at certain times. A critical element in the commercial fruit trade is the inability to store fruit for extended time periods. Kordylas (1990) estimated post-harvest fruit loss to be 20-50% in developing countries. These losses are attributed to a lack of knowledge in fruit handling and marketing. Minimal processing of raw fruits is very important, the essence of processing is to add value to and increase the palatability of fruit. Processing of fresh fruits is necessary, as the fruits´perishability rate is very high, due to lack of cold storage facilities in the rural areas. However, minimally processed foods are good media for growth of microorganisms and represent a potential health risk. The types and counts of microorganisms in minimally processed fruits are affected by the indigenous microflora, the microorganisms contaminating before and after processing, the effects of processing and packing and the intrinsic and extrinsic factors related to the fruit. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1999) have limited to 5 log [CFU/g] the microbial load in minimally processed fruits to consider the product commercially acceptable. The microbial safety and stability as well as the nutritional and sensory quality of most foods are based on an application of combined preservative factors (hurdles). The most important hurdles used in food preservation are temperature, redox potential, water activity, preservatives, modified atmosphere, acidity and competitive microorganisms (Capozzi *et al*., 2009).

Cyrtocarpa procera Kunth is an indigenous Mexican tree that belongs to Anacardiaceae plant family, rarely more than 6 m tall, with twisted limbs, very pale gray bark and pinnate foliage which is native to arroyos in thorn-scrub forest from Jalisco to Puebla and Oaxaca. *C. procera* is used in traditional Mexican medicine (known locally as chupandia or copaljocote), the bark is employed to treat ailments such as diarrhea, dysentery and cough (Argueta *et al*., 1994; Soto and Sousa, 1995). *Cyrtocarpa procera* tree is found growing up to an elevation of

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1500 m. It is an essential nutritious fruit plant as this fruit is tasty and pleasant. The fruit matures in September, is generally round shaped, only about 2 cm in length, purple or yellow to orange at maturity, obliquely obtuse-oblong, styles often persistent, surface often pubescent, exocarp thin, mesocarp fleshy, endocarp bony, with 1-5 opercula; seeds apparently 1, testa with saddle-shaped patch corresponding to the hilum, cotyledons resiform (Mitchell and Daly, 1991). *C. procera* fruits are highly perishable and cannot be stored for more than 96 h at ambient temperature. However, in cold storage (3-4°C) it can be stored for 7 days. It is thus dire need that this fruit should be explored on commercial basis by scientifically assessing its true nutritional values and extending its shelf life through commercially acceptable products. Fruit preservation through processing by combined methods is a suitable combination of various hurdles, which lead to room temperature stable and also low cost fruit (Daza *et al*., 1997). Fruits processed by combined methods can be consumed as if they were fresh or used as components in food formulation such as ice cream, frozen desserts, yogurts, jellies or jams. As far as we know, no works can be found in literature about the proximate composition and the microbiological shelf life of *C. procera* pulp preserved by combined methods.

The aim of this work was to determine the proximate composition of the pulp of *C. procera*, evaluate the effect of combining temperature, water activity and sorbate in the microbiological stability of *C. procera* pulp, to monitor the storage after processing through physicochemical analysis, and assess the validation of a model developed (Gabriel, 2008) to predict the a_w 25°C as a function of pH and °Brix using the actual measure $^{a}a_w$ 25°C in *C. procera* pulp.

MATERIALS AND METHODS

Cyrtocarpa procera fruit was obtained during the month of September in a local market from the community of Acatizapan that belongs to the state of Oaxaca, Mexico. The fruits were transferred to the laboratory in plastic buckets (5 kg/bucket) and selected according to their quality attributes (without damage and in optimal state of ripeness). The sanitation of the process was ensured by personal hygiene and the use of aprons and gloves. All the utensils used were previously sanitized in 200 mg/L on free chlorine. Fruits were washed by immersion in water and given hot water treatment at 95°C for 1 min (Meyer and Paltrinieri, 1990). After blanching, fruits were properly air dried and the pulp was separated from the seeds and the peel with a fruit-peeling machine (Jersa, DRAIOO20). The pulp was used immediately for analysis and processing.

Proximate analysis of *C. procera* pulp: Crude protein, fat and ash, were determined according to AOAC (1997). The moisture content was obtained with a thermobalance (Sartorius, MA45 model), at 100°C for 45 min. The crude fiber content was carried out following procedures described by Kirk (1996). Total carbohydrates content was estimated by difference. Metabolizable energy values (kcal/100 g) were calculated by multiplying the grams of protein, fat and carbohydrates by the factor of 4, 9 and 4 kcal/g, respectively.

Processing of *C. procera* pulp and experimental setup: The experiment was laid out in a randomized 2^3 factorial with three replications. There were three factors-A) the water activity (a_w) of the pulp was lowered from the native value (0.93) to 0.91 by adding sucrose (100 g pulp/60 g sucrose). It has two levels 0.93 or 0.91, B) addition of preservative with two levels (without preservative or with 400 ppm of potassium sorbate) and C) heat treatment with two levels (without heating or heating between 60°C and 65°C for 3 min). As a result of the different processing, 8 treatments were compared (Table 1). Each replication of the treatment is consisted of 50 g pulp. After processing, *C. procera* pulp (50 g) was packaged under air atmosphere in a sterile polyethylene container with twist-off cap. The samples were stored at 25±0.60°C in a fermentation chamber (Industrias Luckie, S.A., Mexico D.F.). Data on shelf life (days), pH, a_w, Total Soluble Solids (TSS) and yeast and mould load were recorded. Data when possible were recorded at first and at 20, 40, 55, 70, 85 and 100 days of storage period.

Table 1: Experimental design for testing combined effects of a_w, antimicrobial and heat treatment in the preservation of *C. procera* pulp

	Particulars		
Treatments	Water activity (a_w)	Antimicrobial (KS)	Heat treatment (H)
T_1	0.93	0	0
T_2	0.91	0	0
T_3	0.93	400 ppm	0
T_4	0.91	400 ppm	0
T_5	0.93	0	60-65°C for 3 min
T_6	0.91	0	60-65°C for 3 min
T_7	0.93	400 ppm	60-65°C for 3 min
T_8	0.91	400 ppm	60-65°C for 3 min

Water activity: The pa_wkit (Decagon Devices, Inc., Pullman, WA, USA) was used to measure the water activity of the samples a 25°C (a_w 25°C). Prior to using the device, the instrument was calibrated using two standards: 6.0 molal NaCl (0.760a_w) and 13.31 molal LiCl (0.250a_w). The a_w 25°C values were obtained with ±0.01 accuracy. Measurements were done in triplicate.

Shelf life (days): The shelf life is a period of time which starts from harvesting and extends up to the start of rotting of fruits (Mondal, 2000).

pH measurement: The pH of samples was measured using a pH-meter (Corning pH-meter 240) at 25°C.

TSS: The total soluble solids were determined using an Abbe refractometer (Vista-C10).

Microbial counts: Yeast and mould counts were using Chloramphenicol Glucose Agar (CGA), according to ISO 7954. Three replicate samples were randomly withdrawn for each treatment. Serial dilutions were prepared by homogenizing 10 g of pulp with 90 ml of 1% sterile peptone water and diluting up to 10^{-4} concentration. Peptone and agar media were purchased from Applichem (Boca Raton FL, USA). Each dilution was plated in duplicate and incubated at 25°C for five days.

Prediction of water activity from pH and °Brix values: The predictive quadratic polynomial equation (1) for a_w 25°C was used to calculate the predicted a_w 25°C ($^{p}a_w$ 25°C) values (Gabriel, 2008) as a function of pH and °Brix values. The a_w 25°C values measured by the a_w meter ($^{a}a_w$ 25°C) were compared with the $^{p}a_w$ 25°C, to assess the predictive performance of the model.

$$a_w\ 25^{o}C = [0.95 + 0.03(pH) + 1.02 \times 10^{-3}\ (^{o}Brix) + 5.21 \times 10^{-4}\ (pH \times {}^{o}Brix) - 3.95 \times 10^{-3}\ (pH^2) - 1.07 \times 10^{-4}\ (^{o}Brix^2)]^{1/2} \quad (1)$$

Validation of the model: The performance indices, accuracy factor (A_f) and bias factor (B_f) were calculated using equations (2) and (3) respectively.

$$A_f = \text{antilog}_{10}\left\{\Sigma\left|\log_{10}\left({}^{p}a_w / {}^{a}a_w\right)/n\right|\right\} \quad (2)$$

$$B_f = \text{antilog}_{10}\left\{\Sigma\left[\log_{10}\left({}^{p}a_w / {}^{a}a_w\right)/n\right]\right\} \quad (3)$$

Where n corresponds to the number of replications employed in the model validation process.

Statistical analysis: The experiment was conducted in Factorial Completely Randomized Design (FCRD) with 8 treatments (2^3). Each treatment was replicated thrice. The results were analyzed for statistical significance using the technique of analysis of variance (ANOVA). The statistical procedures were conducted with Design-Expert 6.0.1.0. (Stat-Ease, Inc., Minneapolis, MN, USA).

RESULTS

Proximate analysis of *C. procera* pulp: The proximate composition of *C. procera* pulp is shown in Table 2. The pulp contains a low amount of carbohydrate (13.41±0.32%), very low protein (1.61±0.13%) and extremely low fat (0.30±0.0%). The values (%) of moisture, ash and crude fiber were 83.62±0.03, 0.68±0.01 and 0.38±0.15, respectively. The metabolizable energy was 62.78±1.80 kcal/100 g.

Table 2: Proximate composition of the fruit pulp of *C. procera*

Constituent	Fruit pulp (%)
Moisture	83.62±0.03
Protein	1.61±0.13
Fat	0.30±0.00
Ash	0.68±0.01
Crude fiber	0.38±0.15
Carbohydrate (by difference)	13.41±0.32
Metabolizable energy, kcal/100 g	62.78±1.80

Values are means±SD of triplicate determinations

Yeast and mould counts: The Table 3 shown the yeast and mould plate counts throughout storage of *C. procera* pulp, preserved by combined methods. Initial counts of yeast and mould in processed *C. procera* pulp were lower than 5 log [CFU/g] for all treatments. The reduction of a_w from 0.93-0.91 entailed little benefit on the microbial stability of the pulp. Treatments with addition of sucrose (T_2, T_4, T_6 and T_8) preserved the processed pulp slightly better from microbial deterioration, but this results are no statistically significant (Table 4, p = 0.1540). The heat treatment of the pulp had an immediate lethal effect on the initial microbial counts. Hence, pulps with heating between 60°C and 65°C for 3 min (T_5, T_6, T_7 and T_8) underwent a reduction in their initial microbial loads ($p<0.05$) of at least 1 log [CFU/g] compared to the rest of the samples. The addition of 400 ppm of KS had the most significant (Table 4, $p<0.0001$) and determinant influence on the product stability. The microbiological stability of pulps preserved with addition of KS (T_3, T_4, T_7 and T_8) had the maximum (100 days) shelf life, independently of the other factors involved. What is more, total yeast and mould populations were dramatically reduced during the first 20 days of storage and were kept always below 4 log [CFU/g] throughout the 100 days of storage of pulps with addition of antimicrobial, except for the pulps with a water activity of 0.93 and not heat treatment (T_3). Under the latter conditions microbial count were kept below 5 log [CFU/g] for at least 20 days and then underwent a progressive decrease, reaching 0 log [CFU/g] at 40 days of storage. Within the other involved factors the heat treatment was the most important one (Table 4, p = 0.0462) affecting the stability of the pulps. Pulps without potassium sorbate, a_w = 0.93 and no heat treatment, spoiled rapidly during the first two weeks of storage (T_1, control treatment). The two or three way interaction between aw, KS and heat treatment not showed significant (Table 4, $p>0.35$) effects on the CFU/g content of *C. procera* pulp during the storage period. These results points out that using low concentrations of KS in combination with other hurdles is a feasible way to preserve processed *C. procera* pulp for long storage periods.

Water activity: During storage, a_w varied from 0.79-0.93, comparisons of means of treatments showed (Table 5)

Table 3: Mould and yeast plate counts (Log_{10} FU/g) throughout storage of *C. procera* pulp

	Storage period (days)							Shelf life
Treatments	0	20	40	55	70	85	100	(days)
T_1	4.79±0.045	7.29±0.133	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	14
T_2	3.80±0.00009	6.96±0.916	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	25
T_3	4.63±1.170	4.16±0.072	0*	0	0	0	0	100
T_4	2.61±0.700	0	0	0	0	0	0	100
T_5	1.55±0.625	6.12±0.014	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	19
T_6	1.16±0.087	4.33±1.080	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	35
T_7	0	0	0	0	0	0	0	100
T_8	0	0	0	0	0	0	0	100

Values are means±SD of triplicate determinations, *0 = no detected (below the detection limit of the plate-counting method 0.5 log CFU/g)

Table 4: Influence of preservation factors on the total yeast and mould counts of *C. procera* pulp

Factor	Effect	Contribution (%)	Significance (p)
a_w	0.81	1.20	0.1540
SK	-6.12	67.90	<0.0001
H	-1.15	2.39	0.0462
(a_w) (SK)	-0.53	0.50	0.3517
(a_w) (H)	0.057	0.00599	0.9188
(SK) (H)	0.46	0.39	0.4130
(a_w) (SK) (H)	-0.34	0.21	0.5443
Pure error	--------	27.40	--------

that maximum a_w were observed in T_1 (control), T_3, T_5 and T_7, all of them at zero days of storage. These treatments correspond to the high level of a_w (native value) of *C. procera* pulp (0.93). The minimum values of a_w were observed in T_4 (0.79) and T_8 (0.86) at 100 days of storage. These treatments correspond to the low level (0.91) of a_w where sucrose was added. The values of a_w for the treatments T_3 and T_7 were the most stable.

Total soluble solids: Table 5 also shows the effect of treatments on changes in total soluble solids (°Brix) of *C. procera* pulp during storage. The TSS varied from 4.61-56.00 °Brix. It was evident from the data that TSS increased significantly throughout the storage period in the T_3, T_4, T_7 and T_8 treatments. Maximum TSS of 56.00 °Brix was reported in T_4 at 100 days of storage. TSS decreased in the treatments (T_1, T_2, T_5 and T_6) that resulted spoiled. Minimum TSS of 4.61 °Brix was reported in T_1 (control) at 20 days of storage.

pH: The pH of *C. procera* pulp varied during storage from 2.44-3.48. The pH in the fresh pulp was 3.44, from (Table 5) it is found that pH of the T_3, T_4 and T_8 treatments decreased during storage ($p<0.05$). The pH of T_7 did not change significantly ($p>0.05$) during storage. Minimum pH of 2.44 was reported in T_3 after 100 days of storage. The pH level of T_1 (control) and T_5 increased from 3.44 and 3.43-3.52 and 3.50 respectively after 20 days of storage and then the samples were spoiled. The pH values in T_2 and T_6 decreased from 3.42 and 3.32-3.35 and 3.30 respectively after 20 days of storage and then the samples were spoiled.

Estimation of water activity as a function of pH and °Brix: Table 6 presents the predicted and observed water activity values and the bias and accuracy factors appropriate to that data for the model proposed by Gabriel (2008), for the estimation of water activity as a function of pH and °Brix. In all the treatments resulted that ${}^{p}a_w$ 25°C>${}^{a}a_w$25°C. The A_f takes values > 1.00, A_f values calculated from model validation using *C. procera* pulp ranged from 1.04-1.14 (range: 0.10). The calculated A_f and B_f values resulted equal. The Fig. 1 shows the graphical comparisons of the predicted and actual calculated a_w 25°C by plotting ${}^{p}a_w$ 25°C (y) against ${}^{a}a_w$ 25°C (x). The Line of Equivalence (LOE) indicate the region of the plot where ${}^{p}a_w$ 25°C = ${}^{a}a_w$ 25°C. The LOE is the line with an equation y = x and diagonally bisects the plot into two equal regions. All the points fell above of the LOE. In the figure can be seen that only six model predictions had % error values greater than 10% (A_f >1.10).

DISCUSSION

The macro components are generally analyzed for their proximate amounts (Owusu-Apenten, 2005). The pulp of *C. procera* was very high in moisture content (83.62±0.03%) and this way underscore its high perishability and susceptibility to microbial infections; and this is indicative of low solid matter in the pulp. The moisture content was within the range of moisture content for fruits and vegetables [60-83 g/100 g, (FAO, 1968)]. The protein content was low (1.61±0.13%), fruits in general are usually not considered as excellent sources of proteins (Ishola *et al*., 1990). The Recommended Dietary Allowance (RDA) for protein is equal to 0.8 g per kg body weight per day (National Research Council, 1989), an adult man of 70 kg requires 56 g of protein daily, assuming complete protein absorption about 3478 g of *C. procera* pulp will satisfy the daily requirement of an adult. The lipid content of *C. procera* pulp was very low (0.30±0.00%); hence the pulp may not be possible source of oil-soluble vitamins (A, D, E and K). Holloway (1983) showed that the composition of fruits and vegetables dietary fiber were predominantly arabinose, galactose and uronic acid, which are water soluble. The low fiber content of *C. procera* pulp (0.38±0.15%) was indicative of be a bad source of dietary fiber. *C. procera* pulp was low in ash

Table 5: Effect of treatments on changes in water activity, pH and total soluble solids (°Brix) of *C. procera* pulp during storage

		Storage period (days)						
Treatments		0	20	40	55	70	85	100
Water	T_1	0.93±0.005	0.92±0.005	spoiled	spoiled	spoiled	spoiled	spoiled
activity	T_2	0.91±0.000	0.88±0.000	spoiled	spoiled	spoiled	spoiled	spoiled
	T_3	0.93±0.000	0.92±0.000	0.92±0.006	0.92±0.005	0.89±0.000	0.91±0.000	0.91±0.000
	T_4	0.91±0.000	0.87±0.003	0.89±0.006	0.88±0.000	0.84±0.000	0.82±0.000	0.79±0.006
	T_5	0.93±0.000	0.92±0.000	spoiled	spoiled	spoiled	spoiled	spoiled
	T_6	0.91±0.005	0.90±0.000	spoiled	spoiled	spoiled	spoiled	spoiled
	T_7	0.93±0.005	0.92±0.000	0.92±0.003	0.91±0.003	0.92±0.006	0.92±0.000	0.92±0.000
	T_8	0.91±0.005	0.90±0.008	0.90±0.009	0.88±0.000	0.87±0.006	0.87±0.010	0.86±0.000
	T_1	3.44±0.09	3.52±0.10	spoiled	spoiled	spoiled	spoiled	spoiled
	T_2	3.42±0.00	3.35±0.00	spoiled	spoiled	spoiled	spoiled	spoiled
	T_3	3.42±0.00	2.79±0.03	2.60±0.03	2.58±0.02	2.53±0.00	2.59±0.01	2.44±0.00
	T_4	3.44±0.00	3.26±0.02	3.28±0.00	3.25±0.05	3.22±0.00	3.23±0.00	3.06±0.01
pH	T_5	3.43±0.00	3.50±0.02	spoiled	spoiled	spoiled	spoiled	spoiled
	T_6	3.32±0.17	3.30±0.08	spoiled	spoiled	spoiled	spoiled	spoiled
	T_7	3.38±0.15	3.30±0.12	3.43±0.06	3.48±0.03	3.45±0.00	3.43±0.00	3.42±0.00
	T_8	3.43±0.11	3.44±0.13	3.28±0.05	3.26±0.07	3.28±0.01	3.23±0.07	3.12±0.04
	T_1	10.14±0.30	4.61±0.05	spoiled	spoiled	spoiled	spoiled	spoiled
	T_2	44.99±0.00	41.83±0.00	spoiled	spoiled	spoiled	spoiled	spoiled
	T_3	10.00±0.00	10.39±0.25	10.83±0.16	10.75±0.09	10.66±0.28	10.80±0.00	13.89±0.53
°Brix	T_4	44.83±0.00	46.50±0.00	49.94±1.80	50.99±0.00	52.61±0.09	55.27±1.17	56.00±6.92
	T_5	10.17±0.00	7.14±0.10	spoiled	spoiled	spoiled	spoiled	spoiled
	T_6	45.83±0.00	45.00±0.16	spoiled	spoiled	spoiled	spoiled	spoiled
	T_7	10.44±0.19	10.61±0.25	10.94±0.25	11.22±0.19	11.05±0.09	11.14±0.23	11.16±0.16
	T_8	47.05±0.45	47.00±0.72	48.83±0.76	48.61±1.45	48.48±0.83	49.49±1.59	50.33±2.01

Values are means±SD of triplicate determinations

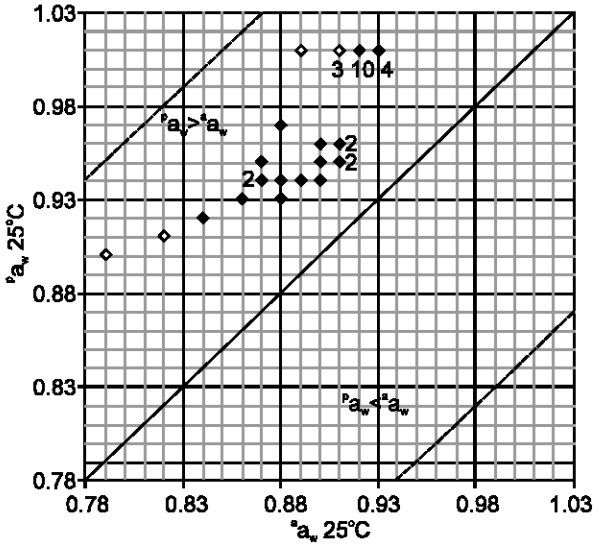

Fig. 1: Graphical comparison between the model-generated $^{p}a_w$ 25°C and actual measured $^{a}a_w$ 25°C in *C. procera* pulp. The numbers placed near the points indicate the number of points that coincided in the same coordinates. The line bisecting the plot is the LOE, while area bound by the dotted lines indicate the ±20% prediction error region where $A_f > 1.20$, ◊ model predictions that had % error values > 10% ($A_f > 1.10$), ◆ model predictions that had % error values ≤ 10% ($A_f \leq 1.10$)

content (0.68±0.01%) and this was indicative of low mineral value, especially the macrominerals. The carbohydrate content of *C. procera* pulp was very low (13.41±0.32%) and this may be responsible for insipid taste. Carbohydrates were the major energy source in the pulp, providing 85% of the energy (53.64 kcal/100 g). The low carbohydrate and gross energy (62.78±1.80 kcal/100 g) values may qualify *C. procera* pulp as a good component in food formulation for the obese and diabetics.

C. procera pulp is a High Moisture Fruit Product (HMFP) and suffers a rapid deterioration after harvest that conducts to a loss of organoleptical quality. Fungal spores and latent infections are either on the surface in the first few cell layers under the peel of the fruit (Barkai-Golan and Phillips, 1991). In order to reduce the initial microbial load by inactivating heat sensitive microorganisms, the *C. procera* fruits were exposed to 95°C for 1 min, blanching has been found to reduce the microbial load from 60-99% (Alzamora *et al*., 1995). In addition, this heat treatment has a sensitizing effect on the survivors, which would be less resistant to the stresses imposed by a_w reduction and by the presence of potassium sorbate.

The pH of the *C. procera* pulp was 3.44 (acid foods have a pH < 4.5) and is not readily spoiled by bacteria but are susceptible to spoilage by yeast and moulds (Wiley, 1997). The initial microflora of the pulp was composed of yeast and mould populations. The initial microbial counts were scarcely diminished by reduction of a_w and

Table 6: Validation of the performance of the predictive model by comparison to *C. procera* pulp

Treatment[A]	Storage period (d)	Food properties[B]		a_w 25°C			Performance indices	
		pH	°Brix	$^{p}a_w$	$^{a}a_w$	Δa_w[C]	A_f	B_f
T_1	0	3.44	10.14	1.01	0.93	0.08	1.09	1.09
T_1	20	3.52	4.61	1.01	0.92	0.09	1.10	1.10
T_2	0	3.42	44.99	0.96	0.91	0.05	1.05	1.05
T_2	20	3.35	41.83	0.97	0.88	0.09	1.10	1.10
T_3	0	3.42	10.00	1.01	0.93	0.08	1.09	1.09
T_3	20	2.79	10.39	1.01	0.92	0.09	1.10	1.10
T_3	40	2.60	10.83	1.01	0.92	0.09	1.10	1.10
T_3	55	2.58	10.75	1.01	0.92	0.09	1.10	1.10
T_3	70	2.53	10.66	1.01	0.89	0.12	1.13	1.13
T_3	85	2.59	10.80	1.01	0.91	0.10	1.11	1.11
T_3	100	2.44	13.89	1.01	0.91	0.10	1.11	1.11
T_4	0	3.44	44.83	0.96	0.91	0.05	1.05	1.05
T_4	20	3.26	46.50	0.95	0.87	0.08	1.09	1.09
T_4	40	3.28	49.94	0.94	0.89	0.05	1.06	1.06
T_4	55	3.25	50.99	0.93	0.88	0.05	1.06	1.06
T_4	70	3.22	52.61	0.92	0.84	0.08	1.10	1.10
T_4	85	3.23	55.27	0.91	0.82	0.09	1.11	1.11
T_4	100	3.06	56.00	0.90	0.79	0.11	1.14	1.14
T_5	0	3.43	10.17	1.01	0.93	0.08	1.09	1.09
T_5	20	3.50	7.14	1.01	0.92	0.09	1.10	1.10
T_6	0	3.32	45.83	0.95	0.91	0.04	1.04	1.04
T_6	20	3.30	45.00	0.96	0.90	0.06	1.07	1.07
T_7	0	3.38	10.44	1.01	0.93	0.08	1.09	1.09
T_7	20	3.30	10.61	1.01	0.92	0.09	1.10	1.10
T_7	40	3.43	10.94	1.01	0.92	0.09	1.10	1.10
T_7	55	3.48	11.22	1.01	0.91	0.10	1.11	1.11
T_7	70	3.45	11.05	1.01	0.92	0.09	1.10	1.10
T_7	85	3.43	11.14	1.01	0.92	0.09	1.10	1.10
T_7	100	3.42	11.16	1.01	0.92	0.09	1.10	1.10
T_8	0	3.43	47.05	0.95	0.91	0.04	1.04	1.04
T_8	20	3.44	47.00	0.95	0.90	0.05	1.06	1.06
T_8	40	3.28	48.83	0.94	0.90	0.04	1.04	1.04
T_8	55	3.26	48.61	0.94	0.88	0.06	1.07	1.07
T_8	70	3.28	48.48	0.94	0.87	0.07	1.08	1.08
T_8	85	3.23	49.49	0.94	0.87	0.07	1.08	1.08
T_8	100	3.12	50.33	0.93	0.86	0.07	1.08	1.08

[A]Only the treatments where was possible to measure the food properties (pH and °Brix)

[B]Values are reported as mean of triplicate determinations

[C]Calculated by subtracting the actual ($^{a}a_w$) from predicted ($^{p}a_w$)

rose rapidly. In our work, the shelf life of untreated pulp (T_1) was found to be hardly 17 days during storage at 25°C. The reduction of a_w from 0.93-0.91 had not significant effect on the stability of the pulp. Moreover, the amount of sucrose needed to get a_w reduction was too high and the pulp resulted too sweet. In addition, Jay (1986) reported that some yeast and moulds, the dominant microorganisms throughout storage, can grow in solutions of 60% sucrose, which limits the use of this hurdle. Many yeasts and moulds are able to proliferate at $a_w < 0.86$, with some osmophilic yeasts and xerophilic moulds capable of slow growth just above 0.6 (Fung, 2009). Several works have demonstrated that heat treatments may reduce the activities of enzymes that are normally enhanced during storage (Sanchez-Ballesta *et al*., 2000; Sapitnitskaya *et al*., 2006; Hirsch *et al*., 2008). In this work we analyzed the possibility of using a combined treatment with heat to delay postharvest deterioration of minimally processed *C. procera* pulp. In the treatments where heat was applied alone or in combination with a_w reduction (T_5 and T_6), there were a significant ($p<0.05$) reduction in the CFU/g in comparison to the control treatment, but was not sufficient to get a good microbiological stability of the pulps. The microorganisms survived the heat treatment, eventually reached the same viable numbers as in the untreated control and spoilage the pulps during storage. It had been demonstrated that reducing the water activity by adding sucrose, increases the resistance of yeast cells to the effects of heat (Beuchat, 1981). The addition of sorbate to the pulps had an immediate lethal effect on the initial microbial counts. Sorbate has been shown to inhibit the growth of yeast, moulds and many bacteria (Sofos and Busta, 1981). Potassium sorbate is

permitted in all countries of the world, since it is considered among the antimicrobial preservatives of low toxicity (Davidson and Juneja, 1990), it can be metabolized similarly to naturally occurring fatty acids. As a result it has received a Generally Recognized as Safe (GRAS) status (FDA, 1978). The pulp of *C. procera* has a low pH (3.44) and the pKa of potassium sorbate is 4.75, this mean that have a higher proportion of antifungal salt in the undissociated form, which is responsible of the antimycotic effect (Gould, 2000). It was obvious from the data that there was an extension of shelf-life (100 days) of *C. procera* pulps when were exposed to 400 ppm of sorbate. Compared to pulps with KS and without heat treatment, pulps with the addition of KS and heat treatment, showed only slight differences in survival of yeast and moulds. Greater lethality, relative to that in pulps without heat treatment was observed. Alzamora *et al*. (1995) and Tapia de Daza *et al*. (1996) observed the same phenomenon in studies with high-moisture fruit products, because the counts of a variety of bacteria, yeast and moulds which survived the mild heat treatment, decreased fast in the products during unrefrigerated storage, since the hurdles applied (pH, a_w, sorbate, sulfite) did not allow growth. It is well documented (Campos *et al*., 1997) that sorbate degrades appreciably as a function of time, temperature, pH and humectants used to depress a_w, during storage of preserved fruits, losing effectiveness as a hurdle. In our work the antimicrobial effect of KS was extended for at least 100 days, this could means that the concentration of KS used may be reduced. Determining the right concentration of a preservative to be used in a food is not easy. Using higher amounts than are needed mean added extra cost to the producer, a negative effect on flavor and possibly a negative effect on health. Using too little preservative has obvious consequences. It was clear that the extent of *C. procera* pulp shelf-life ($p<0.0001$) was mainly due to the addition of KS (T_3, T_4, T_7 and T_8). In T_7 and T_8 the combination with heat treatment contributed ($p<0.05$) to the conservation of the pulps and the addition of sucrose (T_4 and T_8) did not contribute ($p = 0.1540$) to prolong postharvest life of minimally processed *C. procera* pulp. These results also show that, in spite of the determinant influence of heat treatment, pulps can be preserved for 100 days adding only KS (T_3), but the pH of this treatment decreased during storage, from 3.42-2.44 and this affected the flavor significantly. Altogether; the results indicate that the best option is T_7 (native value of a_w, heat treatment and addition of 400 ppm of KS). Moreover, the pH at 100 days of storage for T_7 was 3.42 and did not change significantly during storage, this means that heat treatment and addition of KS were combined to bring additive effect in shelf life improvement and did not entail a negative influence on the sensorial perception of *C. procera* pulp. The pulp of T_7 had excellent visual appearance, flavor and aroma quality after 100 days of storage. In this way it would not be necessary to expend money in the sucrose for depress a_w. The addition of KS and the heat treatment are applicable and affordable for small producers.

The a_w is an important means of predicting and controlling the shelf life of food products. The barrier of a_w change along product storage when sucrose is utilized as humectant. Invertase (ß-D-fructofuranosidase; EC 3.2.1.26) is an irreversible hydrolase and cleaves sucrose into glucose and fructose (Montes de Oca *et al*., 1991). The magnitude of a_w were inversely proportional to the number of storage days, explicitly the longer the pulps were stored, the lower were a_w values. The hydrolysis decreased a_w of the preserved pulps because of the greater capacity of glucose and fructose to reduce a_w. Glucose and fructose have the same a_w lowering capacity (Chirife and Buera, 1996). Moreover, during storage the moisture content of pulps decreased and for this reason a_w may decrease too.

The pulps continue to accumulate TSS during storage (T_3, T_4, T_7 and T_8), the increase could be due to dehydration or hydrolysis of sucrose, because the content of carbohydrate corresponds to 80-95% of the TSS value (Fischer and Martinez, 1999). TSS content may increase also due to the alteration in cell wall structure and breakdown of complex carbohydrates into simple sugars during storage (Rathore *et al*., 2007). When pulp spoilage occurred (T_1, T_2, T_5 and T_6), the TSS decreased substantially, this could be attributable to the respiration process of the pulp (Seyoum, 2002) and microorganisms, the later need the carbohydrate for growth and reproduction (Kays, 2004).

The pH in samples T_1 (shelf-life = 14 d) and T_5 (shelf-life = 19 d) increased, because the only source of carbohydrates was the pulp (13.41±0.32%), there was a poor production of acid, this make the pulps more susceptible to spoilage by yeast and moulds. The pH in samples T_2 (shelf-life = 25 d) and T_6 (shelf-life = 35 d) decreased probably due to decomposition of fermentable substrate especially the carbohydrates in the pulp and the sucrose added to depress a_w, acid production was rapid and low pH slowed down the rate of growth of yeast and moulds (Jay, 1986). The pH of samples T_3, T_4 and T_8 decreased gradually during storage, but the decrease was more in T_3, probably the depressed a_w of samples T_4 and T_8 helped to keep the pH in higher values. The pH of sample T_7 was near the pH of the fresh *C. procera* pulp throughout the 100 days of storage. According to the 2005 version of the Food Code (FDA, 2005), that considers the interaction of a_w and pH in determining if a food is designated as a non-PHF (Potential Hazard Food), the pulp of *C. procera* (T_7) is considered as non-PHF ($a_w > 0.92$-0.95, $pH < 4.6$) throughout the time of storage.

The reliability of the developed model to predict the a_w 25°C as a function of pH and °Brix (Gabriel, 2008), was assessed through validation with the *C. procera* pulp results. A_f values calculated from model validation using *C. procera* pulp ranged from 1.04-1.14 (range: 0.10). Ideally, predictive models should display $B_f = A_f = 1$ (accurate and not biased). From the results of *C. procera* pulp $B_f = A_f > 1$, meaning that the proposed model present little bias, that is, a deviation of values over the LOE. This means that the model overestimates a_w 25°C. Carrasco *et al*. (2006) explained that, a model that forecasts a response from two predictive variables may be expected to have *Af* values that range from 1.20-1.30 or an equivalent % error range of 20-30%. Based on the results obtained from *C. procera* pulp ($A_f < 1.20$), the predictive performance of the established model can be considered acceptable. Six model predictions had % error values greater than 10%. Nevertheless these points were still within a 20% error ($A_f < 1.20$) and hence can still be considered to have acceptable accuracy. As explained by Gabriel (2008), the differences between $^{p}a_w$ 25°C and $^{a}a_w$ 25°C may be due to the influences of food components (carbohydrates, salts, proteins and other soluble components) on the a_w no present in the simulated food solutions used in establishing the model. Results of the validation with *C. procera* pulp showed that the developed model has acceptable predictive performance.

We have demonstrated that shelf life of *C. procera* pulp can be extended up to 100 days at ambient temperature (25°C) without deterioration in quality, by combining practical and inexpensive preservation methods, heating the pulp between 60°C and 65°C for 3 min ($p<0.05$) and addition of 400 ppm KS ($p<0.0001$). The combination of hurdles used was enough to control the growth of yeast and moulds populations that predominate in the indigenous microflora of *C. procera* and due to the low carbohydrate and gross energy values may qualify *C. procera* pulp as a good component in food formulation for the obese and diabetics

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