Changes of total polyphenolics and vitamin C in acerola during storage and spray drying process

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ABSTRACT

Acerola fruit is known to have a high vitamin C concentration. Polyphenolics are also natural oxidants occurring in plants. Understanding changes of these components during storage conditions and processing steps become important. Results of this research showed that there was significant difference in vitamin C and total polyphenolic concentration and three popular varieties of acerola fruits from Tien Giang province. Concentrations of both vitamin C and polyphenolics reduced rapidly during storage at room temperature. After three days, vitamin C reduced about 40% whereas total polyphenolics reduced about 70%. The losses at refrigerated temperature after 3 days were less than 15% and less than 30%, for vitamin C and total polyphenolics, respectively. Frozen storage of the fruit maintained quite well vitamin C and polyphenolics. Acerola pomace juice was concentrated before spray drying and, at the same vacuum pressure, temperatures influenced significantly the retention of vitamin C and total polyphenolics. Optimization of spray drying conditioners including inlet hot air temperatures and added ratio of maltodextrin (drying carrier) was also carried out to obtain high recovery of dry matter, total polyphenolics and vitamin C.

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1. Introduction

Acerola is known as an excellent source of vitamin C (Mezadri et al., 2008). Estimatedly, a cup (180 ml) of acerola compressed juice, containing potentially 35 mg/mL ascorbic acid, is equivalent to the amount of vitamin C of 14 L orange compressed juice (Johnson, 2003). According to Decarvalho & Manica (1994), the concentration of vitamin C in acerola fruit was about 5 – 20 times higher compared to guava, about 10 – 15 times compared to mango. Especially, vitamin C occurs mainly in the pulp of acerola while it occurs at higher concentration in the peel of guava. Concentration of vitamin C in compressed juice of acerola juice is higher than that in compressed juice of oranges, lemons, grapes,... Therefore, acerola fruit could be used as a commercial source of vitamin C for daily diet or a supplement to other foods. As well, acerola juice can be added to other fruit juices to increase the vitamin C content.

Polyphenols are of secondary metabolites widely found in the plant kingdom. These compounds have received great attention nowadays mainly due to their antioxidant potential and the relation between their consumption and the prevention of some diseases associated with oxidative stress, including cancer, and others such as cardiovascular diseases and osteoporosis. Polyphenols found in acerola (*Malpighia emarginata DC.*) include anthocyanins, quercitrin, hyperside, flavonols, astilbin and proanthocyanidin (Hanamura et al., 2005). Rufino et al. (2010) reported 1063 mg gallic acid equivalents/100 g pulp of Brazil acerola. Because of the large amount of vitamin C and polyphenols, acerola has a high antioxidant capacity (Mezadri et al., 2008).

Tien Giang and Ben Tre are two primary plantation areas of acerola in Vietnam and the three varieties are sweet (*Malpighia punicifolia L.*), traditional sour (*Malpighia glabra L.*) and imported sour variety (which is locally called new sour variety) which is also called Brazil (*Malpighia emarginata D.C.*) variety. In different parts of the world, acerola can be processed into powder, juice, applied as vitamin C pills or applied in facial cosmetics... In Vietnam, most of acerola is stored at room temperature for selling as fresh fruit. This storage condition could not be good to preserve natural antioxidants like vitamin C and polyphenols. Processing of the fruit into different products may help increase the value of acerola and the products can be stored for longer time for consumption. It has been known for long time that acerola is a good source of vitamin C, as discussed. Recently, acerola can be also a good source of polyphenols. These components are antioxidants and good for health. However, they are sensitive to processing as well as storage conditions. Therefore, suitable storage and processing conditions should be considered to preserve as much as possible the bioactive components.

Spray drying of acerola juice into powder has a high potential since the powder can be applied in many forms of products; such as pills, cosmetic supplements or instant beverage. Temperature to do spray drying is a critical parameter, and its effects on the retention of the phytochemicals need to be investigated. It seems not possible to spray-dry the juice without adding carriers (maltodextrin, corn syrup, anhydrous starch, gum arabic, whey protein concentrate, whey protein isolate ...). Juice dry matter contains a substantial amount of sugars and the spray-dried products become very sticky, so they easily stick to the wall of the drying chamber and are difficult to be collected. The sugar perhaps also prevents the evaporation of moisture if no carrier is added. The use of carrier may also protect the sensitive components. Therefore, addition of carrier is necessary and more experiments should be done to find out suitable added concentration to give an efficient process.

The objectives of this research are to determine the concentrations of polyphenols and vitamin C in three acerola varieties grown in Vietnam and to investigate the changes of the components during storage, evaporation to concentrate the juice, and spray drying the juice into powder. Experiments were carried out to find suitable conditions to perform those processes with less loss of the antioxidants.

2. Materials and Methods

2.1. Materials and chemicals

Fresh acerola fruits were picked directly in gardens in Go Cong town, Go Cong district, Tien Giang province and were used for analysis or experiments within five hours after picking. The fruits selected were of similar ripeness (just ripened), characterized by a complete maturity, the peel of fruit near the stem was smooth and well out, light green to orange yellow with pink spots, and were hard with no damage due to insect or transportation. Maltodextrin was of Japanese product, in form of white powder with a moisture content of 6-7% and DE value of 20.

For chemicals used for analysis, metaphosphoric acid, acetic acid of $\geq$ 99.98%, thiourea ($CH_4N_2SO_4$), sulfuric acid H_2SO_4 of $\geq$ 99.98%, bromine, ethanol of $\geq$ 99.5%, acid clohydric (HCl), and sodium carbonate were of Chinese products. Other chemicals were 2,4-dinitrophenylhydrazine of $\geq$ 99.5% (Germany), standard ascorbic acid for food of $\geq$ 99.98% (India), Folin-Ciocalteu reagent of $\geq$ 99.8% (Merck, Germany), and standard gallic acid of $\geq$ 99.9% (Merck, Germany).

2.2. Experiments

2.2.1. Determination of concentrations of total polyphenolic compounds and vitamin C in three acerola varieties grown in Go Cong district, Tien Giang province

Fruits of three varieties; namely sweet variety (*Malpighia punicifolia L.*), sour variety (*Malpighia glabra L.*), and Brazil variety (*Malpighia emarginata D.C.*) were the subjects of the analysis. Each variety was picked from three gardens and the whole experiment was carried out in triplicate. All measurements were performed in, at least, duplicate.

2.2.2. Changes of total polyphenolics and vitamin C during storage at various conditions

The experiment was designed to evaluate the effects of storage conditions on the evolution of content of total polyphenolic compounds and vitamin C in acerola fruits. The variety for this experiment was the sweet acerola (Malpighia punicifolia L.). The fresh fruits were put in Styrofoam trays and covered with a PE foil and stored under three different conditions, namely room temperature, 4 ± 2^0C, and freezing at -18 ± 2^0C. Representative samples were taken for analysis of total polyphenolic compounds and vitamin C after 1, 2, 3, 4 and 30 days of storage. The experiment was carried out in triplicate.

2.2.3. Effects of evaporation temperatures on the retention of polyphenolic compounds and vitamin C in acerola pomace juice

Concentration of diluted juice using evaporation before spray drying to obtain powder is more economical in term of energy than direct spray drying of the diluted juice into powder. This experiment was designed to evaluate the effects of evaporation temperatures, performed at the same vacuum pressure, on the retention of polyphenolic compounds and vitamin C.

Frozen sweet variety acerola was thawed and the seeds were removed using a stainless steel knife. The pulp (including the peel) was blended using a multifunction blender (Cornell Inc., USA) and filtered against several layers of a cheese cloth. The pomace juice was fast blanched for 1 minute at 80^0C and standardized at 7% dissolved solids. Each 200 g of the juice was subjected to evaporation to 15% dissolved solids at three different temperatures, namely 65, 75 and 85^0, using a rotary evaporator set at a vacuum pressure of 0.86 ± 0.02 kg/cm^2. The loss of polyphenolic compounds and vitamin C was determined. The experiment was carried out in triplicate.

2.2.4. Optimization of spray drying of the concentrated acerola pomace juice into powder in consideration of hot air temperatures and added ratio of maltodextrin

After screening the effects of hot air temperatures and the added ratio of maltodextrin using one factor experiments, an optimization experiment was carried out to evaluate simultaneously the effects of hot air temperatures and added ratio of maltodextrin on the recovery of dry matter, polyphenolic compounds and vitamin C.

Surface methodology using Central Composite design was applied. Two factors; x_1, hot air temperatures, and x_2, added ratio of maltodextrin (maltodextrin solids/ juice solids) were included with three levels as described in Table 2. The pomace juice was blanched and concentrated to 15% dissolved solids using the rotary evaporator set at 65^0C and 0.86 ± 0,02 kg/cm^2, as described previously, before added with maltodextrin and inspired into the spray dryer. The spray dryer used was a Labplant SD – Basic (Labplant Inc., UK). The operation conditions of the spray dryer were 0.15 ± 0,02 MPa for the compressed air to spray the juice and the input pump was set at 20 mL/min. The fixed settings and experimental parameters were taken in a way that the obtained powders had moisture content of 5.5% and below (3.5-5.5%), to meet the requirement of a stable powder during storage.

The full quadratic equation (Eq. 1) was fit to the obtained data to model the process

$$\begin{aligned} \mathrm{Y_i} &= \mathrm{a_{io}} + \mathrm{a_{i1}}x_1 + \mathrm{a_{i2}}x_2 \\ &\quad + \mathrm{b_{il}}x_1x_2 + \mathrm{c_{il}}x_1^2 + \mathrm{c_{i2}}x_2^2 \end{aligned} \tag{1}$$

Where a_{io}, a_{i1}, a_{i2}, b_{il}, c_{il}, and c_{i2} were regression coefficients and $i = l–3$, representing three responses, namely recovery of dry matter, recovery of total polyphenolic compounds, and recovery of vitamin C.

Recovery yield of dry matter was determined as the percentage of the obtained dry matter in the powder compared to the input dry matters (of the pomace juice and of the added maltodextrin, if used). Similarly, the recovery yield of polyphenolic compounds and vitamin C was the percentage of the components remaining in the obtained powder compared to their amount in the inspired (pumped into the spray dryer) juice.

2.3. Analyses

2.3.1. Chemical analysis

Moisture content or dry matter content of samples was determined using the method of drying to constant weight with drying temperature of 105^0C.

The content of dissolved solids in the juice was determined using a 0 – 32^0 Brix Atago refractometer.

Concentration of total polyphenolic compounds was determined using spectrometry method (UV-VIS 2502 spectrometer, LaboMed Inc, USA) at 700 nm after reaction with Folin-Ciocalteu reagent (Lima et al., 2005). Gallic acid was used as the standard to build the calibration curve. Content of total polyphenolic compounds was expressed as µg gallic acid equivalents (GAE) per gram of sample (pulp in case of analysis of the fruit).

Concentration of vitamin C was determined using spectrometry method (UV-VIS 2502 spectrometer, LaboMed Inc, USA) after reaction with 2-4 DNPH and the absorbance was recorded at 521 nm (Rufino et al., 2010). Ascorbic acid was used to build the calibration curve and the concentration of vitamin C was expressed as μg/g sample (pulp in case of analysis of the fruit).

2.3.2. Data analysis

Average calculation and plotting was performed with Microsoft Excel 2007. JMP software 9.2 (SAS Institute Inc, NC 27513, USA) was used for designing the two-factor experiment and for statistical analysis. The difference was considered significant at the $P < 0{,}05$.

3. Results and Discussion

3.1. Concentration of total polyphenolic compounds and vitamin C in acerola fruits of three varieties grown in Go Cong district, Tien Giang province

Concentrations of total polyphenolic compounds and vitamin C in acerola of the three varieties are shown in Table 1. There was variation in concentrations of total polyphenolic compounds and of vitamin C in the same varieties of different gardens; however, the difference was insignificant. Composition of acerola fruit is known to be influenced by environmental conditions and culturing practices (Mezadri et al., 2005). The fruits selected for the experiments were based on the same ripeness, but this could not be judged exactly by the appearance. Therefore, the variation in polyphenolics and vitamin C due to the difference in ripeness could not be ruled out (Vendramini & Trugo, 2000; Mezadri et al., 2005).

The concentrations of the components of the three varieties were significantly different (Table 1). The Brazil variety was characterized with the highest concentration of total polyphenolic compounds, followed by the sour variety and then the sweet variety. The same trend was observed with the concentration of vitamin C. Rufino et al. (2010) analyzed acerola (*M. emarginata D.C.*) grown in Brazil and reported vitamin C concentration of 1357 mg/ 100 g, which is quite in range with our results.

The results (Table 1) showed that, acerola was not only rich in vitamin C but also in polyphenolic compounds and that this fruit can be a good source for this antioxidant.

3.1.1. Changes of total polyphenol content and vitamin C content during storages at various conditions

The reduction of concentrations of polyphenolic coumpounds and of vitamin C in fruits of sweet variety (Malpighia punicifolia L.) during storage at three different conditions is shown in Figure 1. Concentrations of polyphenolic compounds and vitamin C were reduced during storage and storage conditions strongly influenced the rate of the reduction (Figure 1).

After 30 days of storage at – 18^0C, polyphenolic compounds were reduced of 16.15% while the vitamin C concentration was reduced of 6.29%. Both these changes were statistically significant. The reduction of the components during chilling storage and room temperature storage was much faster. Especially, after three days of storage at room temperature, the vitamin C concentration was reduced of 81.87% and polyphenolic compounds were reduced of 37.51%. It was observed that the fruits became too ripen (rotten) and mold started to grow at 4 days of storage at this condition.

For storage at 4 ± 2^0C, the reduction of both components was observed after each day of storage. After one month, the vitamin C concentra-

Table 1. Concentrations of total polyphenolic compounds and vitamin C in acerola fruits of three varieties grown in Tien Giang province[1]

Variety	Sweet variety (*M. punicifolia L.*)			Sour variety (*M. glabra L.*)			Brazil variety (*M. emarginata D.C.*)		
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8	G_9
Polyphenol (mg GAE/100g)	1153.2 ± 64.7	1195.7 ± 18.9	1295.7 ± 46.0	1441.3 ± 21.3	1336.2 ± 28.6	1226.2 ± 26.6	1563.8 ± 30.9	1429.8 ± 33.2	1534.0 ± 27.2
Average	1214.9 ± 73.2^b			1324.59 ± 107.6^b			1509.93 ± 70.4^b		
Vitamin C (mg/100g)	725.4 ± 7.1	743.1 ± 15.6	762.7 ± 16.2	1226.7 ± 41.9	1093.3 ± 66.8	970.7 ± 32.8	1365.3 ± 35.3	1279.1 ± 18.7	1405.3 ± 17.5
Average	743.7 ± 18.7^c			1096.9 ± 128.0^b			1349.9 ± 64.5^a		

[1]Data are expressed as means ± S.D. G1-9 represents gardens 1 to 9. Three samples of different days were taken for each garden. On the same row, values do not share a common superscript differ significantly.

tion was reduced of 77.26% while polyphenolics were reduced at a less extent of 26,09% (Figure 1). It can be concluded that during chilling storage, the loss of polyphenolics was slower than that of vitamin C. At a long time of storage under this condition, the color of acerola fruits already changed due to water loss.

The results of this experiment pointed out that storage conditions are critical for preservation of the antioxidants in acerola. In reality, e.g., in Vietnam, acerola fruits are displayed at room conditions during selling and this practice should be discouraged.

3.2. Effects of concentration temperatures on the retention of polyphenolic compounds and vitamin C in acerola pomace juice

The fresh juice of sweet variety for this experiment had 7% dissolved solids, and concentrations of vitamin C and total polyphenolic compounds were 1225.78 mg/100 g, 1302.13 mg/100 g, respectively. The fresh pomace juice was blanched at 80 oC for 1 min to inhibit the browning, and then concentrated to 15% of dissolved solids. The effects of evaporation temperatures on the retention/loss of polyphenols and vitamin C are illustrated in Figure 2.

It was observed that, blanching caused loss of polyphenols and vitamin C. Subsequent evaporation caused further loss of the components (Figure 2). At the same vacuum pressure, namely 0.86 ± 0.02 kg/cm2, evaporation at 65^0C retained 70.63% polyphenols and 56.5% vitamin C, compared to amounts occured in the fresh pomace juice. While evaporation at 75^0C and 85^0C retained 60.59% and 51.07% polyphenols, respectively, and 49.55% and 43.73% vitamin C, respectively, although the evaporation time was 5 and 10 min less than that at 65^0C.

It can be concluded that evaporation temperature is an important factor influencing the loss of antioxidants in the acerola pomace juice. It was interesting to note that, the loss of vitamin C was more pronounced than that of polyphenols.

3.3. Optimization of spray drying of the concentrated acerola pomace juice into powder in consideration of hot air temperatures and added ratio of maltodextrin

Two-factor experiment to evaluate the effect of hot air temperatures and added ratio of maltodextrin was carried out, as described previously. The results obtained with all the ten runs of the experiment are shown in Table 2.

Analysis using JMP software showed that, the models in Eq. 1 explained very well the obtained data shown in Table 2, as illustrated that all three responses had $P < 0.01$ and R^2 values of 0.98 and above.

"Parameter estimation" analysis to show the significance of regression coefficients is shown in Figure 3. Coefficients having *P values* < 0.05 were considered as significant and included in the established equations for Y_1, Y_2 and Y_3 (Table 3).

In the zone of experiment, x1 or hot air temperatures (^{0}C), had significant effects, both as linear term or quadratic term, to all the three responses (Figure 3 & Table 3). In the experiment zone, x2 or added ratio of maltodextrin had significant effect as linear term to only recovery yield of dry matter (Figure 3 & Table 3). There was an in-

Table 2. Effects of hot air temperatures and added ratio of maltodextrin on recovery of dry matter, polyphenolic compounds, and vitamin C

Run	Code	x_1 (^oC)	x_2 (w/w)	Dry matter recovery yield (%)	Polyphenols recovery yield (%)	Vitamin C recovery yield (%)
1	- -	130	1.5	84.34	49.54	43.52
2	a0	130	2	84.02	50.97	44.13
3	+ +	130	2.5	83.34	46.36	44.46
4	0a	140	1.5	84.62	54.35	44.84
5	00	140	2	84.55	55.56	46.17
6	00	140	2	84.38	54.48	46.25
7	0A	140	2.5	84.23	55.25	42.11
8	+ -	150	1.5	82.35	30.66	31.30
9	A0	150	2	82.65	40.64	31.40
10	+ +	150	2.5	82.02	44.38	31.22

Table 3. Established regression equations and their peak parameters for the three experimented responses[1]

Response: Recovery of	Established regression equations	Response maximum value	At values of	
			x_1 (^0C)	x_2 (time)
Dry matter (%)	$Y_1 = 84.55–0.78x_1–0.29x_2–1.29x_1^2$	84.81	136.4	1.57
Polyphenols (%)	$Y_2 = 55.83–5.2x_1 + 4.22x_1x_2–10.84x_1^2$	56.60	138.2	2.16
Vitamin C (%)	$Y_3 = 45.46–6.37x_1–6.96x_1^2$	46.93	135.4	1.96

[1] x_1 is hot air temperatures ^0C, x_2 is added ratio of maltodextrin (maltodextrin solids/juice solids).

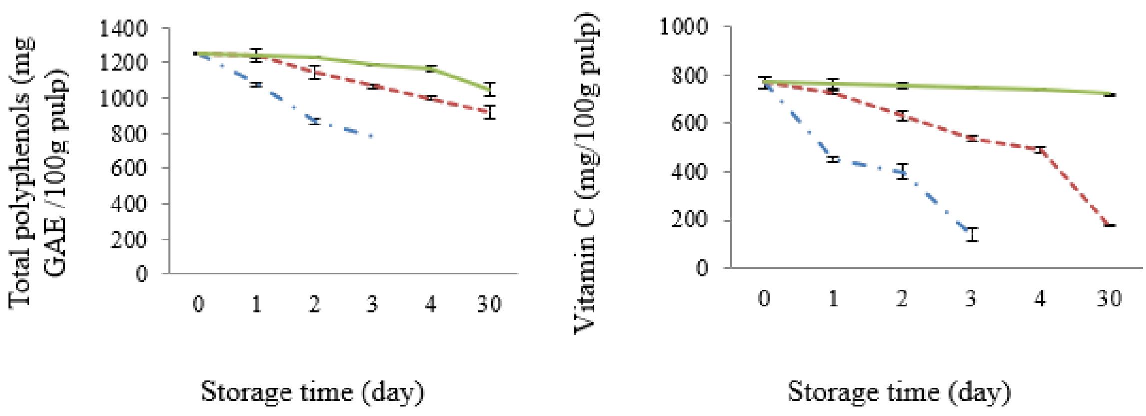

Figure 1. Changes of concentrations of total polyphenolic compounds (above) and of vitamin C (below) during storage of sweet variety acerola fruits at room temperature (–.–), 4 ± 2^{0}C (– –), and -18 ± 2^{0}C (——).

teraction of x_1 and x_2 on the recovery yield of polyphenols. All the three models were quadratic, meaning that the response surfaces were curve ones and maximal values could be inferred.

The spray drying conditions to obtain separately maximum values of the three responses are

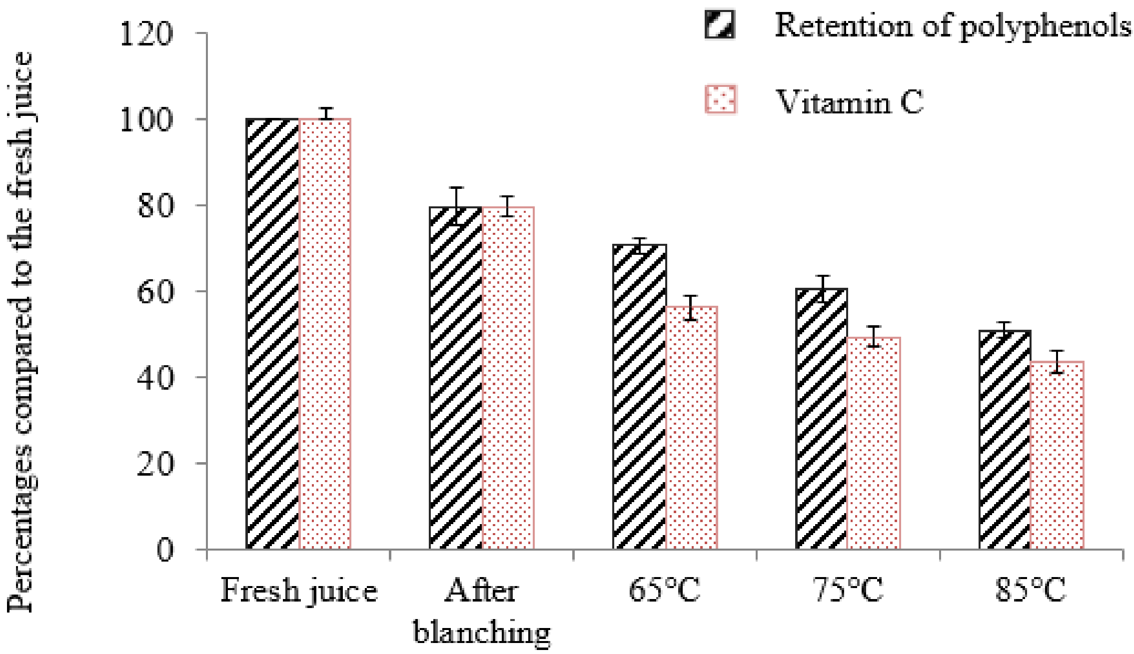

Figure 2. Retention of polyphenols and vitamin C (expressed as percentage compared to the components occurring in the starting material – the fresh pomace juice) under the effects of evaporation temperatures at 0,86 ± 0,02 kg/cm^2.

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	84.545714	0.10015	844.19	<.0001*
X1(130,150)	-0.78	0.068416	-11.40	0.0003*
X2(1.5,2.5)	-0.286667	0.068416	-4.19	0.0138*
X1*X2	0.1675	0.083792	2.00	0.1162
X1*X1	-1.291429	0.109709	-11.77	0.0003*
X2*X2	-0.201429	0.109709	-1.84	0.1402

(a)

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	55.834286	1.017419	54.88	<.0001*
X1(130,150)	-5.198333	0.69503	-7.48	0.0017*
X2(1.5,2.5)	1.9066667	0.69503	2.74	0.0517
X1*X2	4.225	0.851234	4.96	0.0077*
X1*X1	-10.84357	1.114527	-9.73	0.0006*
X2*X2	-1.848571	1.114527	-1.66	0.1725

(b)

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	45.468571	0.800655	56.79	<.0001*
X1(130,150)	-6.365	0.546952	-11.64	0.0003*
X2(1.5,2.5)	-0.311667	0.546952	-0.57	0.5993
X1*X2	-0.255	0.669876	-0.38	0.7228
X1*X1	-6.962143	0.877074	-7.94	0.0014*
X2*X2	-1.252143	0.877074	-1.43	0.2266

(c)

Figure 3. Retention of polyphenols and vitamin C (expressed as percentage compared to the components occurring in the starting material – the fresh pomace juice) under the effects of evaporation temperatures at 0,86 ± 0,02 kg/cm^2.

shown in Table 3. The conditions were quite similar on hot air temperatures but quite different on added ratio of maltodextrin (Table 3). Therefore, setting a drying condition where all the three responses got the maximum values would be impossible. Compromised conditions, as suggested by JMP software, to obtain simultaneously as highest as possible recovery yields of dry matter, polyphenols, and vitamin C were 137.1 – 138.9 oC for hot air temperatures and 2.02 – 2.19 for added ratio of maltodextrin solids compared to juice solids.

4. Conclusions

Experiment results showed that concentrations of polyphenols and vitamin C were different in the three acerola varieties, and the Brazil variety had highest concentrations of both phytochemicals, 1509 mg/100 g pulp for polyphenolics and 1350 mg/100 g pulp for vitamin C. Sour acerola variety was richer in concentrations of the components than sweet acerola variety. Storage conditions influenced the reduction rate of the components. After one month of storage of sweet variety at -18 ± 2^{o}C, polyphenols were reduced by 16.2% and vitamin C reduced 6.3%. These values were actually much smaller compared to the loss of the components during storage at chilling and room temperatures. At room temperature, sweet acerola variety could only be stored for less than 4 days and at three days about 81.9% of polyphenols and 37.5% of vitamin C were lost. At the same vacuum pressure to concentrate juice of 7% to 15% dissolved solids, 0.86 ± 0.02 kg/cm^2, lower evaporation temperatures (65^{0}C was better than high temperatures (e.g., 75 and 85^{0}C in term of retention of polyphenolic compounds and vitamin C, even though the former condition had longer processing time. Hot air temperatures and added ratio of maltodextrin, the carrier, influenced the drying processing efficiency. In the experiment zone (temperatures ranged from 130 – 150^{0}Cand added ratio of maltodextrin ranged from 1.5 to 2.5 times) to spray dry 15% dissolved solids juice, temperatures influenced more pronouncedly to the recovery yields of dry matter, polyphenols, and vitamin C in the obtained powder. The optimal conditions to obtain simultaneously as highest as possible the values for the three recovery yields were 137 – 139^{0}C for temperatures and 2 – 2.2 for added ratio of maltodextrin.

Results of the research confirmed that acerola is rich in both vitamin C, as known for a long time, and polyphenolic compounds. Processing conditions are critical to the loss of these bioactive components. Further research is needed to evaluate the changes of the components during storage of the powder.

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