International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 11, 2018, pp.-6283-6288.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

RETENTION OF COLOUR AND PHYTOCHEMICALS IN STRAWBERRY PULP IN RESPONSE TO THE PULPING METHOD, CHEMICAL TREATMENTS, PASTEURIZATION, AND STORAGE PERIOD

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Received: May 28, 2018; Revised: June 09, 2018; Accepted: June 10, 2018; Published: June 15, 2018

Abstract: Strawberries are the most perishable fruit being very susceptible to mechanical injury, decay, water loss and physiological deterioration. In order to increase its shelf life, Chandler variety grown in Punjab was processed and evaluated for the retention of colour and phytochemicals by opting different methods of preservation. This study evaluated the stability of colour and phytochemicals in strawberry pulp when subjected to four factors, pulping method, chemical treatment, pasteurization and storage period. The fruits were pulped by hot and cold pulping methods and then the hot pulp was categorized into two lots (pasteurized and unpasteurized). Both the lots were preserved by using combinations of class I and class II preservatives. For the stability of anthocyanins and a* value, pasteurized pulp with combination of sugar and citric acid (52°B+0.75%) were found to be the best method for two months. Combination of sugar, sodium benzoate and citric acid (52°B +1000ppm+0.75%) shows superior retention of phytochemical constituents over 3 months of storage at ambient temperature. So, the above-mentioned methods can be used for preservation of strawberry pulp, which can further be processed into value added products.

Keywords: *Strawberry pulp, preservation, anthocyanin, antioxidant, phenol*

Citation: Manpreet Kaur, *et al*., (2018) Retention of Colour and Phytochemicals in Strawberry Pulp in Response to the Pulping Method, Chemical Treatments, Pasteurization, and Storage Period. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 11, pp.- 6283-6288.

Introduction

Strawberry (*Fragaria ananassa*), one of the most popular highly priced fruit worldwide [1,2] posses important place among other berries with 8.1 MMT global production. As stated by U.S. Department of Agriculture, strawberries are the fifth most popular fruit, after bananas, citrus fruits, apples, and watermelons. In India, it is an important fruit crop significantly capturing cultivation in temperate and sub-tropical regions, primarily in the area of Himachal Pradesh, Uttar Pradesh, Maharashtra, West Bengal, Delhi, Haryana, Punjab, and Rajasthan. India exports strawberries mainly to Austria, Bangladesh, Germany, Jordan, and U.S.A. Colour is one of the most important quality attributes and the prime parameter evaluated by consumers. Among berries, strawberries are most popular and attractive to consumers due to unique flavor, texture and red vivid colour [3]. The particular bright red colours of strawberries are due to the presence of health promoting compound anthocyanin; Pelargonidin-3-glucosidealong with the smaller proportion of cyanidin-3-glucoside. These anthocyanin components additionally posses higher antioxidant activity and decrease the incidence of cardiovascular diseases, diabetes, cancer, and arthritis [4] The attractive red colouration associated anthocyanin of strawberries does not prevail during processing and storage. This degradation during processing and storage can damage the colour quality of the finished product [5]. Many factors responsible for least stability of anthocyanins include pH, temperature, light, oxygen, presence of ascorbic acid, sugars, proteins, sulphites, enzymes and metallic ions [6,7]. If the sugars are present at low concentration, they accelerate the rate of anthocyanins degradation. Primarily lactose, fructose, sorbose at lower concentration have greater degradative effects on anthocyanin rather than glucose, sucrose, and maltose. Due to the oxidation of ascorbic acid or Maillard's reaction, furfural and hydroxymethylfurfuralsare produced. These compounds are readily condensed with anthocyaninsresults in the formation of brown compounds that cause discolouration of products.

As a relevant source of bioactive compounds, strawberries contain a high level of diverse phytochemicals and phenolic components belonging to different classes like ellagic acid, ellagitannins, anthocyanins, proanathocyanins, hydroxycinnamic acid derivatives, catechin and fisting flavons known for their potential health promoting properties [8]. These phytochemicals display superior biochemical actions including antioxidant activity, immunomodulating, inhibition of platelet aggregation and anticarcinogenic properties of the fruit [9,10,11,12]. Fruit being delicate and soft textured is highly prone to fungal and bacterial growth. It is also extremely susceptible to water loss, bruising, mechanical injuries and posses a very limited post-harvest life [13,14,15]. The major limiting quality attributes for strawberries are the decay caused by Gray mold Botrytis cinerea [16] and enzymes such as Polyphenoloxidase and Peroxides which cause accelerated deterioration of strawberries during postharvest handling and processing [17,18].Preservation of strawberry as a whole fruit or in the form of pulp/puree during peak harvest season and its utilization by processing industry in the later seasons could be one of the effective ways to make this crop remunerative [19,20]. The use of pulp for the preparation of processed products is famous worldwide which is generally done by two methods of pulping; hot and cold pulping. Prior to preparation of fruit pulp, heating of fruit cause conversion of colourless proanthocyanidins to anthocyanin for its red colour [21]. Distinctive methods used for preservation of strawberry include preparation of jam, jellies and whole fruit canning in syrup. Chemicals preservatives such as sodium benzoate, potassium sorbate are considered superior in the maintenance of good quality during storage of strawberry and its processed products [22,23,24,25]. The previous study conducted by Bishnoi *et al* [26] reported that the sodium benzoate with 500 ppm concentration was most effective in terms to maintain the qualitative characteristics of preserved strawberry pulp up to 2 months whereas the effect of

pasteurization was noted significant. Similarly, Khan *et al* [27] observed that treatment of strawberry with sucrose solution (30° Brix) + Sodium benzoate (0.05%) + Potassium sorbate (0.05%) was superior in terms of physicochemical and organoleptic evaluation. Citric acid was also used by Benhura *et al* [28] for the preservation of mango pulp. As a result of its short shelf life and loss of colour during processing, development of technology for preservation of strawberry products (pulp) and retention of colour and phytochemicals is very vital. So, this study was conducted with the objectives to check the influence of pulping method on physiochemical, phytochemical and antioxidant activity of cold and hot water extracted strawberry pulp and to study the influence of chemical preservatives, pasteurization, and storage period on physiochemical, phytochemical and colour retention of hot water extracted strawberry pulp.

Materials and Methods

Collection of plant material

Fully mature fresh strawberry of Chandler variety was procured from Jagmohan Organic Farm,Amritsar. The collected samples were washed under running tap water. The edible portion of fruit was weighed and chopped for processing into strawberry pulp.

Preparation of pulp

Uniform red vivid, medium size fresh fruits were selected for the preparation of strawberry pulp. The sepals were removed and the fruits were crushed into pulp using the grinder mixer (Philips, Model HL 1632/00)

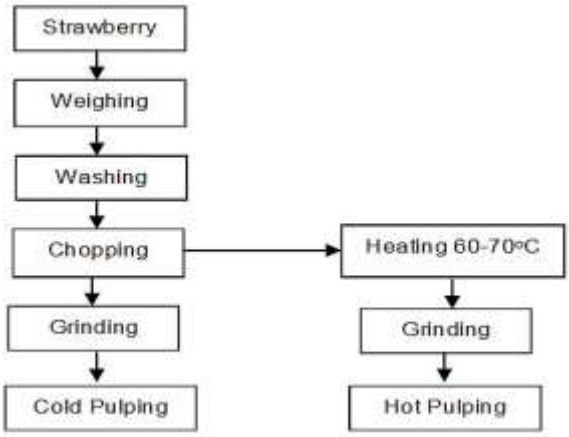

Fig-1 Flow chart for the preparation of strawberry pulp

Chemical treatments

The hot pulp was divided into two lots (Pasteurized and Unpasteurized) and further treated with Class I and Class II preservatives. Nine different combinations were made at different levels according to FDA prescribed limits, as shown in Fig. 2. Samples were stored at ambient temperature up to three months.

No.	Treatment	Level
T_{SP1}	Control#	No preservative
T_{SP2}	CA*	0.75%
T_{SP3}	SB**	2000ppm***
T_{SP4}	CA+ SB	0.75%+1000ppm
T_{SP5}	Sugar	52°B
T_{SP6}	Sugar + CA	52°B+0.75%
T_{SP7}	Sugar + SB	52°B+1000ppm
T_{SP8}	Sugar + SB + CA	52° +1000ppm+0.75%
T_{SP9}	Thermally Treated	100°C/15 min.

*Citric Acid **Sodium benzoate ***Parts per millions
#Control (TSP1) as such hot pulp was used

Physicochemical analysis

For each parameter, samples were analyzed in three replicates. The pH and total soluble solids (°Brix) of the fruit pulp was determined using pH meter (Mettler Toledo) and hand refractometer (Erma, Japan) respectively. Ascorbic acid content was determined by standard titrimetric method described by AOAC 2000using 2, 6-dicholorophenol indophenol dye solution that gets reduced to a colourless compound by ascorbic acid [29]. The total acidity was determined using titration method; 5ml diluted fruit pulp was titrated against 0.1N NaOH using phenolphthalein indicator. The end point was noted (colourless to light pink). The results were expressed as a percentage of citric acid [30]. Colour of the fruit pulp was measured using a Hunter colour lab (Ultra scan, Hunter Lab, USA) in terms of 'L', 'a', 'b' values as described by Buvé *et al* [31]. From these mean values, the total colour change ΔE* was also calculated according to the Equ 1.

ΔE* = √" (L0-L1)2+(a0-a1)2+(b0-b1)2" Equ. 1

The quantification of total sugars and reducing sugars of samples were carried out using Dubois *et al* [32] and Nelson & Somogyi [33,34] methods respectively. Crude fiber was estimated by using a fibertec by acid-base digestion with H2SO4 (1.25%) and NaOH (1.25%) solution (Foss instrument, Sweden).

Phytochemical analysis

The total anthocyanin content was determined by using the pH differential method described by Tonture *et al* [35] with some modifications. The sample of 2gm with a pinch of sodium sulfate grinded with Ethanolic: HCL (85:15) solution until extract became colour-less. The final volume made up to 25ml and quantified spectrophotometrically at 535nm with Ethanolic: HCL solution as a blank. The results were expressed as total anthocyanin (mg/100gm). The total phenolic content of samples was determined using Folin-Ciocalteau reagent according to method described by Singleton *et al* [36]. The free radical scavenging activity was measured by the use of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to the procedure described by Goraya & Bajwa [37].

Statistical analysis

Analysis of variance (ANOVA) was carried out using SPSS (Ver. 16.0) and significant mean differences were compared using Fishers protected least significant difference (LSD) test (5%). Additionally, the t-test was used to test the significance of pulping method on physicochemical and phytochemical constituents of strawberry pulp.

Results and Discussion

Proximate composition and functional components of fresh and processed strawberry

The proximate values of fresh and processed strawberry are presented in [Table-1]. The pulping methods were found to have a non-significant effect on T.S.S, total solids, pH, acidity, total phenols, total sugars, reducing sugars and crude fibre of strawberry pulp. Ascorbic acid content was observed 59.5±0.8 mg/100g of sample in the fresh sample, then which was then reduced to (45±1 mg/100g) during hot pulping. The results were similar to those reported in literature by Ayub *et al* and Zubair *et al* [38,39]. Extension in L, a and bof pulp prepared by hot pulping contrast to cold pulping was observed due to the bleaching of colour components which resulted in increased lightness. The observed results are in conformity with voca *et al* [40]. A significant effect of pulping method on anthocyanin content of strawberry pulp was noticed. A remarkable increase in anthocyanin content (10.6±0.4mg/100g) of pulp with hot pulping is due to the conversion of proanthocyanin (colourless) to bright red anthocyanin. These findings in the present study are identical to previously published reports of Galoburda *et al* [41].

Effect of treatment, pasteurization, and storage on physicochemical properties of strawberry pulp

Chemical treatment, pasteurization and storage study of strawberry pulp significantly affected the total solids, pH, acidity ascorbic acid, antioxidant activity [Table-2]. TSS was observed to decrease in all the nine treatments with respect to the storage period of three months. A corresponding decrease followed by increase in pH value of 9 treatments was observed over the period of 3 months due to the degradation of ascorbic acid; arise in the concentration of weakly ionized acid and salts during storage.

Table-1 Proximate composition and functional components of fresh and processed strawberry

Parameters		Fresh Strawberry	Cold pulping	Hot pulping	Paired t-test
Total solids, %		7.3±0.15	6.8±0.1	6.6±0.11	NS
Crude fibre, %		6.25±0.01	6.22±0.02	6.20±0.01	NS
T.S.S, °B*		6.2±0.1	6.1±0.2	6±0.1	NS
Total sugars, %		6.1±0.05	5.9±0.1	5.7±0.11	NS
Reducing sugars, %		2.8±0.06	3.0±0.2	2.8±0.23	NS
pH		3.56±0.01	3.52±0.01	3.61±0.01	NS
Acidity, % Citric acid		0.31±0.02	0.30±0.01	0.27±0.03	NS
Ascorbic acid, mg/100g		60.2±0.15	59.5±0.8	45±1	S
Total phenol, mg/100g GAE		160±2.51	160±1.5	150±1.5	NS
Antioxidant activity, % inhibition of DPPH		54±1.52	50±1.5	53±2.0	S
Anthocyanin, mg/100g		8.9±0.26	8.6±0.1	10.6±0.4	S
Colour	*L**	33.52±0.02	33.56±0.23	34.22±0.02	S
	*a**	14.62±0.06	14.62±0.12	21.62±0.01	S
	*b**	1.94±0.01	1.94±0.05	7.18±0.02	S

n = 3, Values are Mean ± Standard Deviation; B*= Degree Brix ; S= Significant; NS= Non-significant; L* indicate lightness of the samples; 100 = white, 0 = black; a* designate redness when positive; greenness when negative; b* represent yellowness when positive, blueness when negative

Table-2 Effect of treatment, pasteurization, and storage period on physicochemical properties of strawberry pulp

Treatment	Pasteurization	T.S.S, °B			pH			Acidity, % of Citric acid			Ascorbic acid, mg/100g		
		0 Month	1 Month	3 Month	0 Month	1 Month	3 Month	0 Month	1 Month	3 Month	0 Month	1Month	3 Month
T_{SP1}	PR↑	6±0.15	MG*	MG*	3.52±0.15	MG*	MG*	0.27±0.01	MG*	MG*	45±1.53	MG*	MG*
	UP↑	6±0.15	MG*	MG*	3.53±0.15	MG*	MG*	0.27±0.01	MG*	MG*	45±1.53	MG*	MG*
T_{SP2}	PR↑	6±0.15	MG*	MG*	1.57±0.17	MG*	MG*	1±0.2	MG*	MG*	45±1.53	MG*	MG*
	UP↑	6±0.15	MG*	MG*	1.58±0.15	MG*	MG*	1±0.2	MG*	MG*	45±1.53	MG*	MG*
T_{SP3}	PR↑	6±0.15	5.4±0.2	3.8±0.1	3.52±0.15	3.33±0.02	4.58±0.11	0.27±0.01	1.12±0.02	0.7±0.11	45±1.53	43.5±0.26	34.5±0.26
	UP↑	6±0.15	5.5±0.1	3.9±0.1	3.54±0.16	3.37±0.01	4.65±0.03	0.27±0.01	1.1±0.15	0.65±0.03	45±1.53	44.6±0.21	33.1±0.26
T_{SP4}	PR↑	6±0.15	5.6±0.15	5.0±1	1.57±0.17	1.43±0.01	4.16±0.03	1±0.2	1.9±0.1	1.4±0.15	45±1.53	40.3±0.3	39.8±0.15
	UP↑	6±0.15	5.5±0.15	4.8±0.1	1.56±0.16	1.39±0.01	4.17±0.01	1±0.2	1.8±0.1	1.3±0.2	45±1.53	41.2±0.21	37.8±0.1
T_{SP5}	PR↑	52±1.52	33±1.52	MG*	3.55±0.17	3.30±0.15	MG*	0.27±0.01	1.20±0.2	MG*	45±1.53	41.8±0.1	MG*
	UP↑	52±1.52	34±1.53	MG*	3.52±0.15	3.30±0.20	MG*	0.27±0.01	1.09±0.01	MG*	45±1.53	42.7±0.15	MG*
T_{SP6}	PR↑	52±1.52	41.6±0.15	MG*	1.59±0.18	3±0.25	MG*	1±0.2	0.5±0.11	MG*	45±1.53	42.9±0.1	MG*
	UP↑	52±1.52	41.8±0.21	MG*	1.58±0.15	2.99±0.06	MG*	1±0.2	0.3±0.21	MG*	45±1.53	44.9±0.15	MG*
T_{SP7}	P↑	52±1.52	45±1.53	38±0.1	3.52±0.15	3.53±0.03	4.48±0.12	0.27±0.01	0.17±0.01	0.5±0.15	45±1.53	43.4±0.25	35.5±0.2
	UP↑	52±1.52	47±1	37.5±0.76	3.54±0.16	3.50±0.15	4.45±0.03	0.27±0.01	0.13±0.02	0.7±0.1	45±1.53	44.5±0.29	35.9±0.16
T_{SP8}	P↑	52±1.52	41±1	38±1	1.57±0.17	3.56±0.01	4.03±0.01	1±0.2	0.3±0.2	0.1±0.1	45±1.53	42.3±0.35	36.2±0.1
	UP↑	52±1.52	42±1.53	33±1.52	1.59±0.17	3.54±0.02	4.02±0.01	1±0.2	0.4±0.1	0.2±0.15	45±1.53	44.0±0.58	36.7±0.1
T_{SP9}	P↑	6±0.15	MG*	MG*	3.55±0.15	MG*	MG*	0.27±0.01	MG*	MG*	45±1.53	MG*	MG*
	UP↑	6±0.15	MG*	MG*	3.54±0.15	MG*	MG*	0.27±0.01	MG*	MG*	45±1.53	MG*	MG*
Source							CD (5%)						
A			1.40			0.25			0.54			2.28	
B			0.81			0.14			0.31			1.32	
A×B			2.43						0.93			3.96	
C			NS			NS			0.25			NS	
A×C			NS			NS			NS			NS	
B×C			1.14			0.20			0.44			1.86	
A×B×C			3.43			0.62			0.13			5.60	

A= Treatment; B= Pasteurized or Unpasteurized; C= Storage; n = 3; Values are Mean ± Standard Deviation; MG* = Mold growth; P↑= Pasteurized & UP↑ = Unpasteurized

Results of the present study are in agreement with previous literature [42]. Similarly, the elevation in titrable acidity of preserved strawberry pulp was observed followed by decline over 3 months of storage at ambient temperature. The increase in acidity of strawberry juice preserved with different treatments was as well noticed by Ayub *et al* 2010, in which acidity of samples ranged from 1.33 to 1.44, which was gradually increased to 1.59 to 2.14 per cent, respectively during 3 months of storage. These results are in accordance with the findings of Nunes *et al* [43] who reported an increase in acidity of strawberry during storage, might be due to certain tri-carboxylic acid cycle (TCA) activities, part of sugars being utilized to yield various acids and copolymerization of origin acids. Ascorbic acid is the most inconsistent vitamin which depletes during storage and losses may expand by high temperature, low relative humidity, chilling damage and extended storage. Maximum retention (99.8%) of ascorbic acid was observed in unpasteurized TSP6 sample whereas maximum decrease (10.4%) was observed in pasteurized TSP4 samples. Inactivation of ascorbic acid oxidase and peroxidase by heating during hot pulping also prevent the oxidation of ascorbic acid. Reversible oxidation of ascorbic acid to L- dehydroascorbic acid (DHA) cause the fall down of ascorbic acid content in stored samples. Further irreversible oxidation of DHA generates diketogulonic acid (DCG), which has no biological value as described in Fenemma. Earlier, Merceli *et al* [44] also reported that storage period has a highly significant effect on ascorbic acid contents. After 90 days storage 41.5 per cent reduction of ascorbic acid was notified.

Table-3 Effect of chemical treatment, pasteurization, and storage on colour of strawberry pulp

Treatment	Pasteurization	*L**			*a**			*b**			ΔE*	ΔE
		0 Month	1 Month	3 month	0 Month	1 Month	3 month	0 Month	1 Month	3 month	0-1 Month	0-3 Month
T_{SP1}	PR↑	34.22±0.05	MG*	MG*	21.62±0.04	MG*	MG*	7.18±0.01	MG*	MG*	ND	ND
	UP↑	34.22±0.05	MG*	MG*	21.62±0.04	MG*	MG*	7.18±0.01	MG*	MG*	ND	ND
T_{SP2}	PR↑	34.22±0.05	MG*	MG*	21.62±0.04	MG*	MG*	7.18±0.01	MG*	MG*	ND	ND
	UP↑	34.22±0.05	MG*	MG*	21.62±0.04	MG*	MG*	7.18±0.01	MG*	MG*	ND	ND
T_{SP3}	PR↑	34.22±0.05	35.52±0.02	34.78±0.11	21.62±0.04	8.69±0.16	7.25±0.03	7.18±0.01	5.86±0.03	4.23±0.02	13.06±0.05	14.68±0.03
	UP↑	34.22±0.05	35.50±0.02	33.30±0.05	21.62±0.04	8.73±0.04	7.29±0.02	7.18±0.01	5.89±0.04	4.49±0.06	13.02±0.03	14.61±0.04
T_{SP4}	PR↑	34.22±0.05	35.5±0.02	34.02±0.06	21.62±0.04	4.70±0.15	1.16±0.02	7.18±0.01	1.07±0.02	0.75±0.02	18.03±0.11	21.44±0.02
	UP↑	34.22±0.05	34.40±0.32	33.23±0.04	21.62±0.04	4.69±0.03	1.25±0.02	7.18±0.01	1.09±0.03	0.58±0.02	17.99±0.05	21.41±0.03
T_{SP5}	PR↑	34.22±0.05	44.99±0.01	MG*	21.62±0.04	11.80±0.25	MG*	7.18±0.01	2.60±0.15	MG*	15.28±0.05	ND
	UP↑	34.22±0.05	44.98±0.06	MG*	21.62±0.04	11.78±0.02	MG*	7.18±0.01	2.57±0.25	MG*	15.29±0.03	ND
T_{SP6}	PR↑	34.22±0.05	29.05±0.47	MG*	21.62±0.04	12.10±0.05	MG*	7.18±0.01	4.30±0.1	MG*	11.21±0.02	ND
	UP↑	34.22±0.05	29.15±0.35	MG*	21.62±0.04	12.19±0.03	MG*	7.18±0.01	4.48±0.06	MG*	11.04±0.08	ND
T_{SP7}	P↑	34.22±0.05	28.05±0.05	27.7±0.17	21.62±0.04	6.02±0.03	3.74±0.02	7.18±0.01	2.60±0.15	2.30±0.15	17.39±0.03	19.86±0.04
	UP↑	34.22±0.05	28.25±0.05	27.1±0.04	21.62±0.04	6.16±0.02	3.78±0.02	7.18±0.01	2.78±0.05	2.35±0.03	17.15±0.03	19.84±0.04
T_{SP8}	P↑	34.22±0.05	26.80±0.1	24.06±0.04	21.62±0.04	6.01±0.03	3.57±0.02	7.18±0.01	1.70±0.15	1.03±0.01	18.13±0.03	21.61±0.04
	UP↑	34.22±0.05	27.47±0.02	25.09±0.03	21.62±0.04	6.02±0.04	3.59±0.03	7.18±0.01	1.82±0.01	1.09±0.01	17.82±0.03	21.11±0.04
T_{SP9}	P↑	34.22±0.05	MG*	MG*	21.62±0.04	MG*	MG*	7.18±0.01	MG*	MG*	ND	ND
	UP↑	34.22±0.05	MG*	MG*	21.62±0.04	MG*	MG*	7.18±0.01	MG*	MG*	ND	ND
Source						CD (5%)						
A			0.34			0.28			0.38			
B			0.16			0.16			0.22			
A×B			0.48			0.48			0.66			
C			NS			NS			NS			
A×C			0.48			0.39			0.54			
B×C			0.16			0 .22			0.31			
A×B×C			0.68			0.68			0.93			

A= Treatment; B= Pasteurized or Unpasteurized; C= Storage; n = 3; Values are Mean ± Standard Deviation; MG* = Mold growth; P↑= Pasteurized & UP↑ = Unpasteurized; ND= Non-detectable L* values indicate lightness of the samples; 100 = white, 0 = black; a* values designate redness when positive; greenness when negative; b* values represent yellowness when positive, blueness when negative; ΔE*= Total colour difference

Table-4 Effect of chemical treatment, pasteurization, and storage on phytochemical constituents of strawberry pulp

Treatment	Pasteurization	Anthocyanin, mg/100g			Antioxidant activity, % inhibition of DPPH			Phenol content, mg/100g GAE		
		0 Month	1 Month	3 month	0 Month	1 Month	3 month	0 Month	1 Month	3 month
T_{SP1}	PR↑	11±0.6	MG*	MG*	47.4±0.42	MG*	MG*	150±2.1	MG*	MG*
	UP↑	11±0.6	MG*	MG*	47.4±0.42	MG*	MG*	150±2.1	MG*	MG*
T_{SP2}	PR↑	11±0.6	MG*	MG*	47.4±0.42	MG*	MG*	150±2.1	MG*	MG*
	UP↑	11±0.6	MG*	MG*	47.4±0.42	MG*	MG*	150±2.1	MG*	MG*
T_{SP3}	PR↑	11±0.6	6.7±6.7	1.4±0.15	47.4±0.42	21.2±0.11	8.9±0.06	150±2.1	170.7±0.25	106.7±0.51
	UP↑	11±0.6	6.9±6.8	1.9±0.11	47.4±0.42	20.8±0.25	9.6±0.35	150±2.1	173.3±0.30	110.7±0.68
T_{SP4}	PR↑	11±0.6	4.0±0.5	1.1±0.15	47.4±0.42	30.6±0.15	13.1±0.21	150±2.1	234.0±1	126.7±0.56
	UP↑	11±0.6	4.2±0.15	1.5±0.15	47.4±0.42	29.9±0.61	13.7±0.2	150±2.1	233.3±0.85	124.7±0.68
T_{SP5}	PR↑	11±0.6	8.8±0.1	MG*	47.4±0.42	21.9±0.06	MG*	150±2.1	220.0±2.51	MG*
	UP↑	11±0.6	9±0.5	MG*	47.4±0.42	21.5±0.26	MG*	150±2.1	200.0±4.93	MG*
T_{SP6}	PR↑	11±0.6	10.3±0.2	MG*	47.4±0.42	29.2±0.43	MG*	150±2.1	120.0±5	MG*
	UP↑	11±0.6	10.7±0.15	MG*	47.4±0.42	28.5±0.26	MG*	150±2.1	121.3±0.85	MG*
T_{SP7}	P↑	11±0.6	6.08±0.03	1.87±0.03	47.4±0.42	28.1±0.26	7.5±0.21	150±2.1	194.0±2.08	86.6±0.21
	UP↑	11±0.6	6.11±0.02	2.09±0.60	47.4±0.42	27.7±0.2	8.2±0.17	150±2.1	193.3±1.82	96.7±0.15
T_{SP8}	P↑	11±0.6	8.12±0.04	2.12±0.02	47.4±0.42	33.9±0.26	17.4±0.15	150±2.1	148.0±1	126.7±1.07
	UP↑	11±0.6	8.15±0.01	2.17±0.01	47.4±0.42	33.2±0.3	16.7±0.30	150±2.1	147.3±0.36	130.7±0.68
T_{SP9}	P↑	11±0.6	MG*	MG*	47.4±0.42	MG*	MG*	150±2.1	MG*	MG*
	UP↑	11±0.6	MG*	MG*	47.4±0.42	MG*	MG*	150±2.1	MG*	MG*
SOURCE					CD (5%)					
A			0.26			86.48			87.71	
B			0.15			49.93			50.64	
A×B			0.46			149.79			151.91	
C			NS			NS			NS	
A×C			NS			NS			NS	
B×C			0.22			70.61			71.61	
A×B×C			0.65			211.84			214.84	

A= Treatment; B= Pasteurized or Unpasteurized; C= Storage; n = 3; Values are Mean ± Standard Deviation; MG* = Mold growth; P↑= Pasteurized & UP↑ = Unpasteurized

The minimum loss was observed among the samples stored at refrigeration temperature. Many variables such as pH, temperature, light, oxygen and presence of metallic catalyzers easily degrade the vitamin C. Decomposed compounds reacts with amino acids and form hydroxymethylfurfurals [45,46].

Effect of chemical treatment, pasteurization, and storage on the colour of strawberry pulp

Colour is one of the most important quality attribute asit enhances the acceptance and appeal of a product. [Table-3] compares the colour values of preserved strawberry pulp over 3 month's storage. There was a marked difference between L* a*&b* of all 9 treated samples. L value which designate the lightness of the product, increased significantly. Degradation of anthocyanin and bleaching of pigment components resulted in enhancing L value. Maximum lightness was observed in TSP3 (35.52±0.02 and 35.50±0.02) followed byTSP4 (35.50±0.02 and 34.40±0.32). In contrast, the a* value of the strawberry pulp significant reduced as a function of chemical treatment, pasteurization and storage period. This is in accordance with the results of Garzón and Wrolstad and Wang *et al*. [47,48]. Maximum retention of a* value was noticed in TSP6 samples (12.19±0.03) may be due to fixation of colour by sugars and lower water activity; followed byTSP5 (11.78±0.02) after one month storage. As mentioned in the previous literature by Durge *et al* 2013, one per cent of citric acid retains 25 per cent of anthocyanin that provide redness to pulp [49]. The total colour difference (ΔE*) between 0, 1 and 3 months storage was calculated with [Equ-1]. The change in ΔE* value as a function of treatment, pasteurization and storage period shelf-life is shown in [Table-3]. The change in ΔE* value was clearly depending on the treatment, pasteurization, storage period and was only due to the decrease in a* value.

Effect of chemical treatment, pasteurization, and storage on phytochemical constituents of strawberry pulp

The colour of strawberry depends chiefly on the presence of water soluble anthocyanin pigments. Acidic conditions are helpful for the maintenance of anthocyanin while normal processing and prolong storage cause the transfer of anthocyanin to insoluble brown pigment. The anthocyanin content decreased significantly in all the nine treatments of strawberry pulp from 0 to 3 months storage [Table-4]. Maximum oxidation of anthocyanin was noted in TSP4. The foremost causes of degradation of anthocyanin are cleavage of covalent bonds and oxidation of anthocyanin due to thermal processing. Stability of anthocyanin also influenced by a number of factors such as; pH, light, oxygen, enzymes, structure, and concentration of the anthocyanin, the presence of ascorbic acid, sugars, sulfite salts, metal ions and co pigments [50,51]. Fortification of product with ascorbic acid is a common method to protect them against oxidation [33].

Antioxidant activity of chemically preserved strawberry pulp (expressed as percentage of free radical scavenging activity) was influenced by interaction between chemical treatment, pasteurization and storage period. TSP8 pulp had highest antioxidant activity may be due to the oxidation of OH group of citric acid rather than OH group of compounds that are contributing to antioxidant potential, followed by TSP4, TSP6 at 3 months of storage at ambient temperature. Generally during the storage, antioxidant activity of pulp decreased. Pasteurization, used for the preservation of pulp, can induce undesirable changes such as thermal degradation of thermo sensitive compounds. However, this fact was not observed in present study according to collected data, since pasteurized pulp had higher antioxidant activity than the unpasteurized pulp. The results of present study provide conformity with results of Goncalves *et al* [52]. During a study on apple pulp, Nisar *et al* [53], reported a significant effect of chemical and thermal treatments on antioxidant activity and total phenolic content of samples. The level of total phenolics of strawberry pulp was influenced by preservation treatment, pasteurization and storage period. Due to the release of monomeric compounds from polymeric polyphenol and tannins hydrolysis, significant increase of total phenols of preserved samples was observed in TSP3, TSP4, TSP5 and TSP7 over one month storage. Although, the bioactive compounds losses notified during 3 months of storage. This was partially attributed due to decrease of anthocyanins, phenolics derived from shikimic acid and malonic acid metabolism. During prolong storage and pasteurization, reduced level of total phenolic content was also observed by Oliveira *et al* [54]. The similarity was found with results of Siah *et al* [55] as significant reduction in the total phenol content after 2 months storage of strawberry was observed. Ayala-Zavala *et al* [56] reported that both temperature and storage time had a significant effect on total phenolic compounds of strawberry fruit as total phenolic compounds increased continuously in berries at 5 to 10^{o}C, whereas at 0^{o}C total phenol value remains constant.

Conclusion

Strawberry is a rich source of a wide variety of nutritive compounds. Due to the high respiration rate and high susceptibility to the pathogen attack, strawberry fruit is highly perishable. Along with its high nutritional value, preservation of strawberry and its processed products is the vital necessitate of food industry. The present study shows that hot pulping method has significant positive effect on colour and anthocyanin content of strawberry pulp. Out of the 9 treatment, Tsp6 (pasteurized) shows superior retention of anthocyanin and a* value over 2 months storage, whereas Tsp8 shows better retention of phytochemical constituents over 3 months storage at ambient temperature. So, the present study was conducted to help the fruit processing industries to utilize this fruit for product preparation with increased shelf stability and consumer demand. It can also reduce the post harvest losses and thus it can be available to the customers all the year round.

Application of research

Strawberry as a perishable fruit loss its color and flavor during its processing. From the industrial point of view, this research work was conducted to commercialize the product with retention of color and phytochemicals.

Research Category: Fruits Processing.

Acknowledgement / Funding: Author thankful to S. Jagmohan Singh Nagi, MD of Golden Grains Inc. Verka, Amritsar, Punjab-India. Author also thankful to Punjab Agricultural University, Ludhiana, 141004, Punjab, India

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Research project name: MSc Research

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript
Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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