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Hot Pepper viii. Reduction of Microbial Spoilage and Physio-chemical Deterioration in Processed Caribbean Peppers

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Abstract

The quality in the small producers' hot pepper industry is affected by microbial and thermal processing which reduces the physio-chemical qualities of the final product particularly colour retention and to a lesser extent pungency. A series of studies explored the causes and evaluated some protocols to reduce microbial infection and the loss of pigment using varying sealant caps of vegetable oil and sodium chloride, in addition to preserving agents (calcium chloride, ascorbic acid, sodium benzoate, sodium chloride, and calcium citrate). The result confirmed that red pepper mash in 100g $CaCl_2$ (calcium chloride) submerged under vegetable oil sealant cap retained "L", 'a', 'b' colour coordinates as the fresh peppers beyond 100 days with no loss of colour, pungency and flavour.

Keywords: microbial infection, physio-chemical, capsanthin, hot peppers

1. Introduction

The Caribbean has become renowned for its colourful and flavourful hot peppers (Scotch bonnet) and the infamous Trinidad Scorpion and Carvalho hot which are ranked amongst the world's hottest peppers (*Capsicum chinensis* L.). There is more than one dozen such highly pungent peppers grown in the Caribbean for either fresh consumption or processing. It is very competitive and risky for small producers to trade in the fresh hot pepper markets in the USA and Europe. As a consequence, it is necessary to seek alternative approaches to combat the two major challenges that the industry is plagued with viz: microbial spoilage and colour degradation. Further, the quality of processed hot pepper products such as flakes, mash and sauces are also affected by physio-chemical deterioration. The quality of these value–added products particularly colour, consumer acceptance, and to a lesser extent pungency are reduced. Bridgemohan et al. (2018a, b) improved colour retention in flakes and mash in several Caribbean pepper varieties, but these are still at an unacceptable level.

With respect to colour, the pigment content (chlorophylls, anthocyanin and carotenoids *viz*; capsanthin, capsorubin zeaxanthin, lutein, and cryptocapsin, and α and β carotene) increased as the fruit ripened until post maturity (Mohamed & Bridgemohan, 2014 a &b). The mature fresh fruits under ambient conditions had a shelf life of 4 - 5 days with minimal loss of quality, which could eventually be extended up to 14-15 days at non-chilling temperature regimes (Bridgemohan et al. 2017a; Mohammed et al., 2014). Similarly, immediately after harvest, storage and transportation losses due to physiological, biochemical and microbiological activities could be reduced significantly (Mohammed et al., 2016).

Several flavour compounds were identified in fresh pepper and pepper mash *viz*: 2-pentanone, 3-hexanol, acetic acid, oleic acid, and linoleic acid. However, the compounds (E)-2-undecenal, farnesol, 2-pentadecyn-1-ol, linolenic acid, and squalene are found only in the fresh pepper and not processed products. This suggested that the observed browning reactions in the mash could be an interaction of physio-chemical and microbial degradation, but it is yet to be confirmed (Gogus et al., 2015).

Spoilage in pepper mash may be induced by exposure to heat and faeco-oral route infections, or changes to metabolic processes resulting in undesirable or unacceptable sensory characteristics (Koh 2005). If pathogens or toxins are involved, there are undesirable changes in texture, aroma, taste, or appearance. More often, spoilage is induced by exposure to air, microorganisms (bacteria, yeasts and molds), or improper storage conditions (Bridgemohan et al., 2018a; 2017a, b).

Microbial spoilage in hot pepper products can be visible as mold growth embedded in a mass of filaments with brown colour changes due to the bacterial acidification caused by *L. mesenteroides* (Koh, 2000), *Listeria spp. (L. gelidumsubsp. gasicomitatum, L. piscium* (Pothakos, Vasileios et al., 2014), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Micrococcus luteus,* or *Escherichia coli* (Natheer, 2010), and *Clostridium perfringens* and *Staphylococcus aureus* (Draughton et al, 1981). Flagan & Leadbetter (2006) showed that the reduction in flavour and pungency maybe caused by the growth and utilization of capsaicin nutrient by certain bacteria (*Pseudomonas* and *Variovorax* ***spp***).

The physio-chemical changes are observed in the top layers as brown pigments. This maybe both pH and temperature dependent. Increasing pH (3 to 4) can affect the rate of non-enzymatic browning reactions as a function of temperature (Gogus & Sami, 1998). Flores et al. (2007) noted that while fermentation of chili peppers is dependent on several factors including microbial flora, non-desirable microbial growth could be controlled using $CaCl_2$ treatments. Lee, Jang & Hwang (2002) found that plastic films with appropriate gas permeability, such as co-extruded multilayer polyamide, may be the most practical choice based on consideration of both volume expansion and quality retention of the fermented paste.

Vega-Gálvez (2009) observed that dry conditions, particularly temperature, leads to pepper modifications that can cause quality degradation. The radical scavenging activity showed higher antioxidant activity at higher temperatures (80 and 90 ℃) rather than at lower temperatures (50, 60 and 70 ℃).

Steaming and gamma irradiation are found to enhance the physio-chemical and microbiological properties of dried red pepper during post-treatment storage at a refrigerated temperature (4 ±2 ℃) (Rico et al., 2010). The application of chlorine dioxide (ClO_2) gas treatment can be a potential effective method of pathogen reduction for *Escherichia coli* O157:H7 (Han et al., 2000).

This study is part of a series of experiments which evaluated two approaches to reduce the microbial contamination and pigment deterioration in processed Caribbean peppers. The major issues in processing of red and yellow hot peppers are loss of colour and pungency, and microbial spoilage. The University of Trinidad and Tobago (UTT) in partnership with the major pepper producers and the Export Marketing Agency, undertook the responsibility to assess colour stability and retention of pungency in processed products of selected Caribbean hot peppers destined for the export trade. Consequently, the objective of this study is to determine the effects of preserving agents on the physio-chemical changes on colour stability and shelf-life as well as the reduction of microbial contamination in hot peppers produced in the Caribbean.

2. Materials and Methods

This study was conducted during the period 2014-2018. Experiments were conducted at the laboratories of the St. Augustine Campus, University of the West Indies and at the Biosciences, Agriculture and Food Technology, University of Trinidad and Tobago. All the peppers used in the study were cultivated at the Waterloo Research Campus Field Station, and only if necessary, procured from reputable farmers for consistent quality and colour. The crop agronomy and postharvest treatments, as well as the methodology used in the mash preparation and sealant caps were previously described and reported by Bridgemohan and Mohammed (2018). The varieties used based on colour were Trinidad scorpion (Red) and Scotch bonnet (yellow). Three separate studies were conducted which addressed the following parameters of colour stability, microbial infection and discoloration, and enhanced mash quality.

Study 1. Effects of preserving agents [Pa] and capping [Sc] on colour retention

The pepper mash was processed using freshly harvested and sanitized (250 ppm sodium hypochlorite] cv. Carvalho hot peppers. Two (2) kg samples of the puree or mash were topped with one litre of the preserving agents [Pa]; acetic, citric acid, and ethanol. To the acetic and citric acids 5g sodium benzoate were added, while no preservatives were applied to the control. The sealant or caps [Sc] were developed using wax paper layer which were placed on top of the mash to eliminate cross-contamination. The Sc evaluated were 500ml of vegetable oil and 100g NaCl, and a control [zero cap]. This experiment covered a period of more than 500 days. All samples were keep in the dark.

Changes to fruit colour were monitored monthly using a portable tristimulus Minolta Chromameter (Model

CR-200, Minolta Corp, Ramesy, NJ). The meter was calibrated with a white standard plate (Minolta calibration plate CR-A43) and fruit chromaticity was measured in "L", 'a', 'b' coordinates.

Study 2. Effect of Sealant Cap (Sc) and Preserving Agent (Pa) on microbial infection (MI) and discoloration (%)

The methodology used in the mash preparation and sealant caps were similar to the above study, and the varieties used based on colour were Trinidad scorpion (red) and Scotch bonnet (yellow). The Sealant Caps (Sc) were oil, salt, no cap, and the Preserving Agents (Pa), included citric acid, alcohol, acetic acid, sodium benzoate, and control (Table 1). The jar size used was 500 ml, and each treatment was replicated 10 times. Changes in colour and spoilage were monitored over a 2-year period.

Table 1. Preserving agent **[Pa]** treatments used in the study.

Preserving agent [Pa]	Code
Sodium Chloride	NaCl
Sodium Benzoate	NAB
Calcium Chloride	$CaCl_2$
Ascorbic Acid	ASC
Calcium citrate	CALCTIR
Citric acid	CA
Acetic Acid	ACA

Study 3. Effect Preserving Agent (Pa) on physio-chemical and microbial infection (MI) on pepper mash quality

This study advanced the results of Study 2, and only the vegetable oil sealant cap [Sc] was further evaluated using both red and yellow hot peppers, and 5 Preserving Agents [Pa]. The Pa included calcium chloride, ascorbic acid, sodium benzoate, sodium chloride, and calcium citrate. There were two controls that had no Pa, but one was not sanitized. This allowed for the observation of the movement of field microbes into the pack house operations. In addition to changes in colour pigments, the pH and total soluble sugars [TSS] were measured, and the level of microbial infection was monitored on fortnightly basis for a period of 100 days. The experiment was a completely randomized block design with 2 colours [Co] and 5 persevering agents [Pa] with five replicates.

All data were subjected to generalized linear modelling (GLIM) using Minitab Statistical Software, and where necessary, variables were first subjected to log transformation and were then analyzed. For all comparisons, significance was defined at $p \leq 0.05$.

3. Results and Discussion

Study 1. Effect of Preserving Agent (Pa) and Sealant Cap (Sc) on colour (Co) stability

All the preserving agents (Pa), regardless of the sealant cap (Sc) did not improve the quality of the mash which resulted in a significant loss in colour or pigment degradation. In the red peppers, there was a marked reduction in L (21%), a* (79%), and b (67%), compared to the yellow for L (45%), a*(72%) and b (82%)] after 100 days (Table 2). The regression analysis of the main factors (Sc, Pa and Co) and its various interactions on L, revealed that only Sc was significant ($P \geq 0.05$), [Equation 1]. Further, the oil cap sealant performed better than both salt (NaCl) and no cap for both yellow and red peppers (Table 2).

$$Y_{L^*} = 37.7 - 2.39\ Sc + 0.01\ Co - 0.85\ Pa + 0.24\ Sc^*Co^*Pa + 0.17\ S^*Co:\ R^2 = 85.87\%. \quad (1)$$

Of the colour variables (L, a* b* and Hue angle), only, L and a* appeared to be affected by the preserving agents and sealant caps. The a* for red coloured peppers appeared to have been influenced more significantly ($P \geq 0.005$) compared to yellow with interactions of sealants, preserving agents and colour (Equation 2).

$$Y_a^* = 14.5 + 0.40\ Sc - 4.96\ Co - 0.05\ Pa + 0.28\ Sc^*Co^*Pa - 0.47\ Sc^*Co^*Pa:\ R2 = 80.0\ \%. \quad (2)$$

The higher retention of red colouration over yellow was very noticeable as evidenced in the ANOVA presented hereunder:

Predictor	Coef	SE Coef	T	P
Constant	14.50	3.58	4.04	0.000
Sc	0.40	1.24	0.32	0.751
Co	-4.95	1.58	-3.13	0.005***
Pa	-0.05	0.81	-0.07	0.946
Sc*Co*Pa	0.28	0.21	1.27	0.218

Generally, acetic acid [ACA] seemed to be the more effective Pa in the maintenance of colour stability compared to the others for both pepper colour in the oil sealant cap (Table 2).

Study 2. Effect of Sealant Cap (Sc) and Preserving Agent (Pa) on microbial infection (MI) and discoloration (%)

The effect of the Sc and Pa and its various interactions on MI were subjected to the GLIM analysis, and the best fit (Equation 3) indicated that only the main factor Sealant Cap [Sc] had any significant ($P \geq 0.005$) effect.

$$Y_{MI} = 22.2 + 0.1\ Co + 1.62\ Pa + 0.13\ Sc: R^2 = 53\% \quad (3)$$

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sc	3	9163.5	9871.9	3290.6	5.63	0.005***
Pa	4	4630.6	4668.5	1167.1	2.00	0.132
Co	1	50.2	50.2	50.2	0.09	0.772

Generally, the mean microbial infection (3.3%) was significantly lower than the salt treatment (36.9%) and no cap (42.5%). Also, in red peppers there was no effect of Pa or Co on microbial infection. The colour of the fresh pepper appeared to be easily subjected to discoloration, regardless of the preserving agent (Eqn 3). The GLIM ANOVA revealed the discoloration was affected by Co and Sc interactions as presented hereunder:

Source	DF	Seq SS
SC	3	9163.48
Pa	4	4630.62
Co	1	50.21
Sc*Pa*Co	15	3707.71
Sc*Pa	11	7968.35
Pa*Co	7	115.50
Sc*CO	5	0.00

The mean discoloration of oil caps sealant (25.9%) was significantly lower than both salt and no cap (42.5%) treatments. Further analysis indicated that there was no correlation between microbial infection and discoloration. Generally, the red pepper treatments with no Pa under oil Cap had zero spoilage. With the exception of alcohol preserving agent, all the Pa in oil Cap prevented microbial infection for both colours unlike the other treatments.

Model Reduced

Predictor	Coef	SE Coef	T	P
Constant	20.29	1.40	14.44	0.000
Co^2	-3.70	0.37	-9.83	0.000**
Pa^2	0.11	0.07	1.55	0.130
Pa	1.22	0.70	-1.73	0.091*
Co*Pa	0.25	0.25		0.323

The effect of the Pa in oil Sc treatments after 100 days revealed significant variations between L (1.642 ± 0.504) for all Pa treatments (Table 3), and L value for yellow peppers were higher than red and increased with Pa resulting in significant interactions [Equation 4]. The converse was observed for a*, with an interaction between colour and preserving agents [Equation 5], where the a* for red pepper was significantly higher than all Pa (14.96 ± 0.727) compared to that of the yellow pepper ($4.85 + 0.825$). The b* displayed a significant quadratic response for both Co and Pa with no interaction [Equation 6] and its b* also varied significantly between Pa (11.2 ± 0.742).

Table 2. The effect of the Preserving Agents in Oil cap treatments after 100 days

Y	Response	R^2	Equation
Y_{L^*}	$39.2 + 2.65\ Co^2 + 0.47\ Pa^2 - 1.84\ Pa - 1.04\ Co^*$	73.1	4
Y_{a^*}	$20.3 - 3.71\ Co^2 + 0.113\ Pa^2 - 1.23\ Pa + 0.25\ Co^*Pa$	90.7	5
Y_{b^*}	$1.76\ Co^2 + 0.582\ Pa^2 - 4.64\ Pa + 13.8$	48	6

Study 3. Effect Preserving Agent (Pa) on physio-chemical and microbial infection (MI) conditions on pepper mash quality

Table 3. Colorimetric Readings on Red and Yellow Pepper Mash

Colour	Treatment	L*	a*	b*
Red	ASC	39.71	12.91	4.35
	$CACl_2$	37.39	16.87	11.86
	CALCTIR	44.44	17.04	13.51
	Control	36.64	15.59	10.02
	NAB	38.02	11.16	3.58
	NACL	42.11	15.45	9.14
	Unsanitized	39.94	15.69	8.09
Yellow	ASC	43.23	4.66	12.38
	$CACl_2$	42.76	5.50	15.29
	CALCTIR	46.81	4.53	18.16
	Control	45.12	4.35	16.35
	NAB	38.92	5.13	6.04
	NACL	42.67	6.01	13.94
	Unsanitized	45.24	3.80	15.25
	X	43.53	9.9	16.7
	se	3.077	5.41	4.34

There were no significant changes in L for red mashes regardless of the Pa**,** but in the yellow mashes there was a 25 to 40 % decrease in L values for all the Pa treatments (Table 3). The a* and b* for both red and yellow mashes deteriorated by >50%, and there was no treatment effect. The microbial infection was affected by both pepper colour and the preserving agent [Equation 7].

$$MI\% = -23.0 + 6.3\ Co + 3.57\ Pa - 0.11\ Co^*Pa.\ R^2 = 85.42\% \quad (7)$$

The infection was due to either bacteria or fungi, but no evidence of cross infections or contaminations were visible. It was observed that the microbial [Bacteria] infection (42%) for red mash was lower than yellow mash (61.5%). The MI due to fungi (10%) was less and the same for both colour mashes (Table 4). The ASC, CACL, CALCITR and NACL exhibited higher bacterial infection (44 to 100%) compared to the sanitized control (29%) in the Yellow mashes. All of the Pa's with the exception of CALCITR reduced the MI spoilage between 0 to 15%. The $CACl_2$ provided good protection against both bacterial and fungal infection in red peppers. The major bacterial were the gram negative rods (Table 5).

Table 5. The Effects Preserving Agent (Pa) on microbial infection (MI) and physio-chemical conditions on pepper mash quality

Colour [Co]	Preserving Agent [Pa]	Microbial			Physio-chemical	
		% Spoilage [MI]	Gram Stains	Motility Agar Test	pH	^{o}Brix
Yellow	ASC	100	+, rods	-	4	8.8
	NAB	36.3	-, rods	+	4	8.3
	$CACl_2$	44	-, rods	-	5	18.3
	NACL	100	-, rods	-	4	4.9
	Control	29	-, rods	+	5.4	6.24
	CALCTIR	85	-, rods	-	4	4.6
Red	ASC	2	-, rods	+	4	8.8
	NAB	15	-, rods	-	4	8.3
	$CACl_2$	0	0	0	4	18.3
	NACL	2	-, rods	-	5	18.3
	Control	25	-, rods	+	4	6.4
	CALCTIR	29	-, rods	+	4	7.8

Generally, the yellow pepper mash was more susceptible to physio-chemical changes (72.5%) compared to red (25%). The two variables monitored were pH and ^{o}Bx, and it was observed that the Pa had no effect on the pH and varied between 4 to 5.4. The $CaCl_2$ treatment increased the ^{o}Bx to 18.3 in both colours, and was similar NaCl for red only. The ^{o}Bx was generally low for all treatments (4.9 to 8.3) and was affected by Pa (Equation 8).

$$Y\ ^{o}_{Bx} = -\ 2.1\ +\ 9.24\ Pa:\ R^2\ =\ 72.43\% \quad (8)$$

There were no changes in the pH (4.3 +- 0.82) in all of the various mashes regardless of colour and preserving agents. The $CaCl_2$ has proven to enhance the shelf-life of peppers by controlling the development of physiological disorders eg., reducing browning (Samira et al. 2013; Yildiz, 2018). It improved the stabilization on the membrane systems and the formation of calcium-pectate which increased the rigidity of cell walls and inhibited degradation (Mignani et al., 1995). This made the pepper cell walls less accessible to the enzymes that could promote softening and cellular leakages (Stanely et al., 1995). The $CaCl_2$ reduced the formation of the brown pigments as the pH was not affected, thus suppressing the non-enzymatic colour changes as suggested by Gogus and Sami (1995).

The ^{o}Brix contents of the of intensely green mature peppers varieties ranged from 3.5 to 6.43 (Mohammed et al., 1999; Antoniali et al., 2007). However, it was observed from this study that the $CaCl_2$ treatment increased the ^{o}Brix from 4.9 and 8.3 to 18.3. Getenit et al. (2008) confirmed that ^{o}Brix contents of stored peppers can result in faster conversion of starch into water-soluble sugars which was facilitated with the $CaCl_2$. According to Antoniali et al. (2007) the polysaccharides of the cell wall are broken up with consequent increases in sugar levels during ripening.

After 100 days the pH in the study varied between 4.0 to 5.4 , which are slightly more acidic than that reported by Medlicott et al.(1986). The reported pH values in the range of 5.99 showed an increasing trend during storage time. The observable changes in pH could be due to variations in citric, malic and ascorbic acid concentrations since these acids are known to diminish during ripening.

Antoniali et al. (2007) also reported that there were no significant differences in pH values of pepper during ripening. However Medlicott et al. (1986) had previosly confirmed there was a tendency of increasing pH values and reduced acidity with prolonged storage time, since the fruit preceeding the ripening process is going to diminish its predominant malic acid. According to Mizrach et al. (1997), carbohydrate and acid metabolism are closely connected during postharvest ripening period which would thus raise pH of the produce pungency.

The processed mashes retained a high level of pungency and appeared to be higher than results of Gibbs & O'Garro (2004) for mature fruits. During ripening, capsaicin concentration reached a maximum then it turned over and degraded to secondary products (Bernal and Barcelo, 1996), thus confirming why the mash may not be as pungent as the fresh fruits. It is assumed that peroxidases catalyze capsasinoid oxidation and plays a central role in its metabolism in pepper (Yu et al., 2005). Peroxidase activity is lower in fruits which have low moisture content (Bernal and Barcelo 1996). Lower peroxidase and capsaicin oxidase activity means that the oxidation, or breakdown, of capsaicin will be slower when fruits have low moisture.

4. Conclusion

The study indicated that red hot pepper mash had better post processing qualities with lower deterioration and physio-chemical changes. Further, the addition of $CaCl_2$ as preserving agent in the red pepper mash, inhibited microbial infection and growth for both bacterial and fungal organisms. The Trinidad scorpion red pepper was able to maintain the aroma, consistency and pungency / taste as in the fresh state. The preserving agents maintained the °Bx at 18.3 which was similar to the fresh state, in addition to colour retention. It can be concluded that for export purposes, the red pepper preserved with $CaCl_2$ under the oil cap sealant can be recommended to reduce spoilage and maintain integrity of post processed peppers.

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Appendix

Table 1. Effects of preserving agents and capping on colour stability

		Red colour peppers				Yellow colour peppers		
Sealant cap	Preserving agent	L*	a*	b*	hue	L*	a*	b*
Oil	1 Citric Acid	36.16	7.69	13.48	60.29	35.23	6.00	13.44
	2Control	36.24	6.59	13.29	63.62	36.24	6.59	13.29
	3Alcohol	33.46	10.03	6.10	31.30	35.59	5.76	13.3313.4
	4Acetic Acid	31.83	11.79	6.54	29.01	36.16	7.69	13.48
	5Sodium Benzoate	31.35	9.08	6.21	34.36	32.75	5.81	10.57
Salt	Citric Acid	31.50	10.36	6.49	32.06	36.02	3.19	4.26
	Control	32.67	6.31	6.35	45.18	32.24	5.69	8.66
	Alcohol	31.09	8.44	5.82	34.58	33.29	4.45	6.41
	Acetic Acid	33.43	5.63	6.16	47.57	35.39	6.46	11.84
	Sodium Benzoate	30.96	7.12	5.84	39.35	32.24	5.31	6.70
zero	Citric Acid	32.01	16.92	9.63	29.64	26.57	2.73	0.49
	Control	31.37	11.40	6.37	29.19	37.86	6.53	14.93
	Alcohol	32.65	9.58	6.34	33.49	33.63	6.26	7.59
	Acetic Acid	35.95	5.76	13.33	66.63	34.84	7.46	14.83
	Sodium Benzoate	28.95	8.85	5.15	30.19	33.02	6.46	11.44
	X [SE]	33.2 [0.479]	7.8[0.594]	8.7 [0.743]	29.01	34.07 [0.689]	5.75 [0.358]	9.86 [1.15]

Table 2. Effect of the cap x treatment on microbial infection (%)

Colour	Treatment	**Caps**					
		Salt		Oil		**No cap**	
		microbial infection	Discoloration	microbial infection	Discoloration	microbial infection	Discoloration
Yellow	Citric acid	9	42	6	10	23	11
	Alcohol	50	46	0	47	82	75
	Acetic acid	34	51	0	33	100	39
	Sodium benzoate	0	63	5	10	23	40
	Control	71	71	0	10	8	15
Red	Citric acid	0	10	0	33	35	6
	Alcohol	68	15	12	24	30	43
	Acetic acid	44	39	10	54	44	24
	Sodium benzoate	8	55	0	38	61	22
	Control	85	35	0	0	19	15
		36.9 [10.01]	42.70 [6.10]	3.3 [1.48]	25.90 [5.69]	42.5 [9.38]	29.0 [6.54]