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Microbiological evaluation of watermelon juice treated with serendipity berry (*Dioscoreophyllum cumminsii*) extract

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Summary

Watermelon (*Citrullus lanatus*) fruit is among numerous fruits that has a high moisture content and is therefore desirable for human consumption. In order to ensure its availability all through the year, processing into juice and other valuable processes are being carried out in order to reduce the rate of spoilage and watermelon juice losses. The high moisture content in fruits makes them highly susceptible to spoilage. Watermelon juice and extract of serendipity berry (*Dioscoreophyllum cumminsii*) were blended together in the following ratios: sample A (100 ml+10 ml), sample B (100 ml+20 ml), sample C (100 ml+30 ml), sample D (100 ml+40 ml), and sample E (100 ml+50 ml) respectively, while pure watermelon juice served as a control sample. Microbial and sensory analyses of the samples were evaluated over twelve weeks of storage. Over the period of storage, the results showed that microbial load of the control sample ranged between $1.1x10^5$-$9.7.6x10^7$ cfu/ml, while treated samples ranged between $0.2\ x10^{5-}\ 1.4x10^5$ cfu/ml, with some of the treated samples having negligible growth <10 cfu/ml**.** Results from the study confirmed that pure watermelon juice was highly susceptible to a microbial attack due to the absence of a preservative, and large volume of microbial loads was recorded, while the treated samples that included serendipity berry extract, which functioned both as a sweetener and a preservative, had fewer microbial loads over same period of storage. The sensory evaluation result showed that sample C (100 ml watermelon juice + 30 ml of serendipity berry extract) was rated the best in terms of taste, aroma and general acceptability, while sample D (100 ml watermelon juice + 40 ml of serendipity berry extract) was rated highest for colour.

Keywords: juice, serendipity berry, sweetener, preservation, microbial load

Introduction

Watermelon (*Citrullus lanatus)* variety of the family *Cucurbitaceae* is a vine-like (scrambler and trailer) flowering plant that originates from southern Africa. It is a large, sprawling annual plant with coarse, hairy pinnately lobed leaves with white to yellow flowers; a special kind of berry botanically called pepo is also grown for its edible fruit (Renner et al., 2007). The fruit, which is usually consumed fresh, led to the rejection of watermelons that have any form of visible defect (Schaefer et al., 2011). Watermelon fruit has a high moisture content (Erukainure et al., 2010) which makes it highly susceptible to microbial spoilage caused by gram positive bacteria. It should, however, be noted that all foods, even when properly packaged, undergo biochemical, physical, and other changes that can affect their quality and safety (Sam and Micha, 2009). Some researchers in their various research works isolated some organisms from the fruit. Escherichia coli and *Klebsiella* species were reported to be isolated from water melon juice (Nwachukwa et al., 2008), while Buchet (1995) reported that *E. coil* was found to contaminate fruit and vegetables. In addition, the ability of these organisms to survive in acidic juices, at both ambient and refrigerated temperature, and low pH value, was documented by Nester et al. (2001).

Physiological and biochemical forms of spoilage in food products may be delayed or prevented by various methods of preservation; such methods are usually aimed at retaining the nutritive value of the product, extending its shelf life and keeping it safe for consumption (Singleton, 1997). Recently, consumers' demand for foods with long shelf life, high quality and affordable price has increased, and to meet these needs, food industries/producers are in search of the improved processing methods and technologies that could decrease damages to fresh, nutritious, and healthy foods. It was stated by Fish et al. (2002) that watermelon is a gastronomically pleasing food and its rich source of lycopene makes it a highly desirable source of phytochemicals. Lycopene is a carotenoid that gives the red colour to tomatoes, watermelons, and red bell peppers, among other fruits and vegetables. Lycopene is a powerful antioxidant and has been shown to prevent various cancers, and it also helps in the prevention of heart disease (Rao and Agarwal, 1999).

Juice is a liquid extract naturally contained in fruit and vegetables. It is commonly consumed as a beverage or used as an ingredient, or flavouring in foods. Blending

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of fruit/vegetable juices is a common practice (Ryan A. Ward, 2011), and most fruit juice industries store their juice by treating it with chemical preservatives in other to inactivate microorganisms and enzymes. This preservative method can cause detrimental effects on the juice quality. Chemical preservatives may cause colour change, separation of particles, and a change in flavour, and/or smell (Qin et al., 1995).

Sweeteners can be either natural or synthetic, and they have been known for their usage as preservatives. The search for natural sweeteners and preservatives has prompted intensive research on plants with sweetening properties. The fruit, serendipity berry, contains a protein sweetener called monellin that could replace sugar in foods for diabetics and dieters (Oselbe and Nwankiti, 2005). Serendipity berry (*Dioscoreophyllum cumminsii*) is among the unpopular and under-utilized fruit found in the forest towards the end of the raining season. The watermelon could be compared with other fruits used in the fruit industry for juice and concentrates production. This fruit could be used in wine and juice production (Abiodun et al., 2014), and could assist in aiding the availability of juice of various fruits, even at their off season.

This research work, however, tends to look at the effect of the sweetening and preservative potency of the extract of the berry on the fruit juice.

Materials and methods

Healthy ripe watermelon fruits were purchased at a local market in Ilorin, Kwara State, while fully matured Serendipity berry fruits were obtained from Oke-Oro farm at Esa-Odo area of Osun State in Nigeria. The laboratory works were carried out within three departments (Department of Home-Economics and Food Science, Department of Chemistry and Department of Microbiology) of the University of Ilorin, Kwara State, Nigeria.

Extraction of watermelon and serendipity berry juice

The raw fruits were washed thoroughly in the running tap water. The thoroughly washed fruits were then peeled and de-seeded. The juice from the watermelon fruits was extracted using a sterilized fruit blender. The extracted juice was filtered through double muslin cloth. Distilled water was added to the peeled serendipity berry, and the gel/juice (sweetener) was extracted in a mixer and filtered through double muslin cloth to obtain the fresh sweetener. The extracted sweetener was blended with the watermelon juice in the ratio stated in Table 1, and stored in sterile plastic containers. The blended samples, pasteurized at 100 °C for 3 minutes, were stored in a cool condition for the period of storage.

Determination of total bacterial and mould counts

Bacterial and mould counts were determined by pour plate technique of Adegoke (2000). 1 g (w/v) of the sample was dissolved in 1ml of 2% sterile sodium citrate solution in order to prepare a suspension. 1 ml of the suspension was then used for the serial dilution of between 10^{-1} to 10^{-6}. 1 ml of the diluted sample was placed in a sterile disposable petridishes (sterilin) in tripicates. At about 44 °C to 50 °C, the various media were poured on to the samples in the petridishes and allowed to set, after which they were inverted and incubated for 48 hours.

Sensory evaluation

Sensory evaluation was conducted on the fresh water melon juice samples (both treated and control) bya panel of 20 untrained personnel who are regular consumers of watermelon. The panelists were directed to rate the samples on the bases of colour/appearance, taste, aroma and overall acceptability. Nine-point Hedonic Scale, ranging from like extremely (9 points), to dislike extremely (1 point), was used.

Statistical analysis

SPSS v.16.0 was used to statistically analyze the obtained results based on the analysis of variance (ANOVA) in order to determine the significant difference of the samples. The means were separated using the Duncan test.

Table 1. Blending of watermelon juice with serendipity berry extract

Samples	Watermelon Juice (ml)	Serendipity Berry Extract (ml)
A	100	10
B	100	20
C	100	30
D	100	40
E	100	50
CTR	100	0

Legend: *SAMPLE A- 100 ml Watermelon + 10 ml of serendipity berry; SAMPLE B- 100 ml Watermelon + 20 ml of serendipity berry; SAMPLE C- 100 ml Watermelon + 30 ml of serendipity berry; SAMPLE D- 100 ml Watermelon + 40 ml of serendipity berry; SAMPLE E- 100 ml Watermelon + 50 ml of serendipity berry; CTR- Watermelon Juice only (Control)*

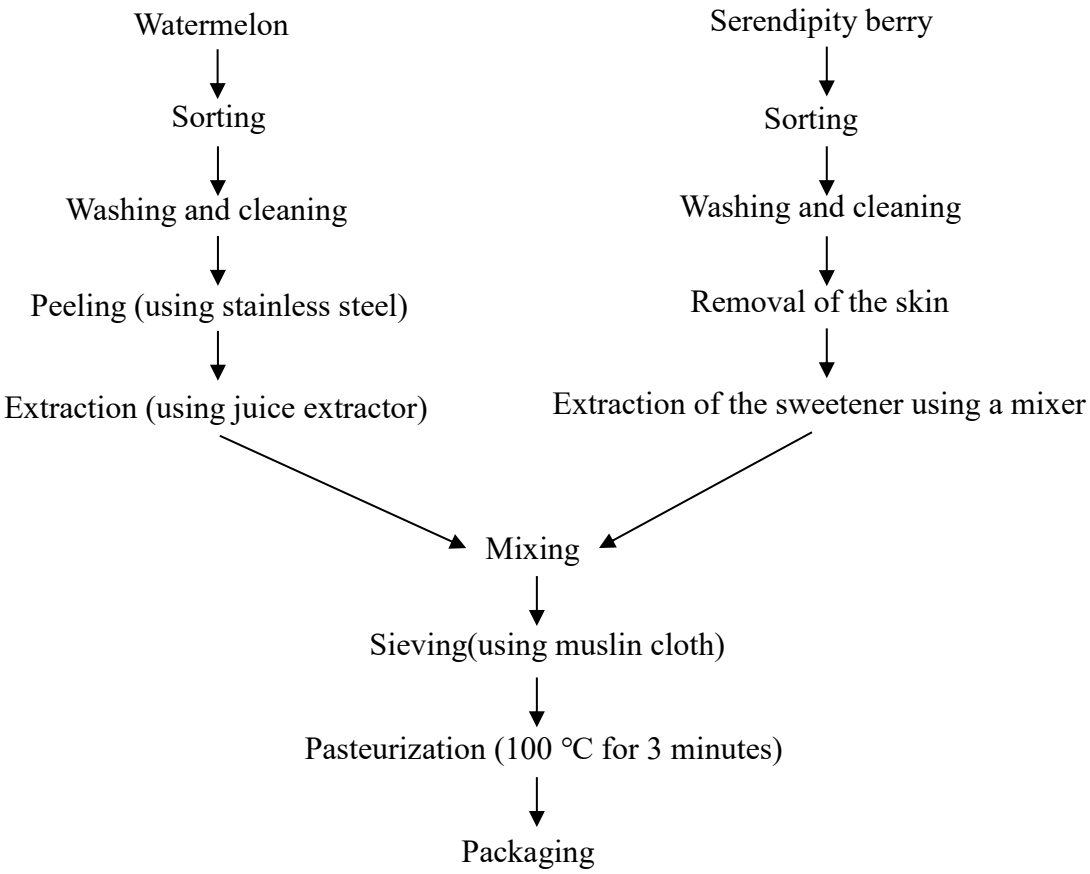

Fig 1. Flow chart showing the production of watermelon and serendipity berry juice

Results and discussion

Microbial analysis

Microbial examinations are usually used as monitoring indices of food spoilage. The results of the microbial analyses of the blend of watermelon and serendipity berry juice extract during storage are shown in tables 2, 3 and 4 respectively, and the colonies were counted in unit per millilitre (cfu/ml) using a hand held colony counter. The total microbial count for all the samples on the nutrient agar (NA), mcConckey agar (MA) and potato dextrose agar (PDA) was minimal at the end of the first week, indicating that the pasteurization of the samples was effective in reducing the microbial loads that might have accumulated during processing or from the environment, thus making the product safe for consumption. Samples C, D and E had <10 cfu/ml on the NA, while in the control sample growth slightly above that recorded for the treated samples was observed. The result was similar to that reported by Ankush et al. (2015). At the end of the storage period, there was a slight microbial and mould growth of the treated samples, when compared to the control samples. The increase noticed in the microbial loads of the treated samples at the end of the storage period was 0.2×10^5 - 1.4×10^5 cfu/ml, while, over same period, the control sample had a large increase of 1.1×10^5 - 9.7×10^7 cfu/ml. Ashaye et al. (2007) reported a reduction in microbial count and a resultant preservation of cheese when treated with *A. danielli* extract. Ceylan et al. (2004) also noted a reduction in the population of *E. coli* (0157:H7) from 5.31 log cfu/ml to 2.2 log cfu/ml in 3 days in apple juice treated with potassium sorbate. The reduction in microbial load could be attributed to the action of the extract of the serendipity berry added to the samples. These values were within the safe limit for juices, as they have not exceeded the standard values of 1.0×10^4 cfu/ml recommended by Ihekoronye (1985). The shelf life of the treated samples was established taking into consideration the microbial population of the untreated sample at the end of the first and twelveth week.

Sensory evaluation of the juice samples

The sensory evaluation result shows that treated samples were rated highly than the control sample for the colour, taste, aroma and general

acceptability of the juice samples. Colour is an important sensory characteristic on which consumer preferences are dependent. Francis (1995) stated that colour influences other sensory characteristics, which subsequently account for food acceptability, choice, and preference. The sensory evaluation result shows that the treatment reflected in the scores/ratings of the samples. Sample D received the highest rating in terms of colour/appearance. The values obtained for sample D showed that it was significantly different from sample E and slightly than the other samples, but technically/statistically, there were no significant differences among them. When the food is chewed, taste receptors in the mouth are activated. While colour/appearance may be the initial quality attributes of a fruit or vegetable product, the taste may have the largest impact on acceptability and desire to consume more. Sample C was preferred mostly in terms of taste, and it was significantly different from samples A and E. Statistically or technically, there was no significant difference between sample C and samples B, D and the control sample, but it had higher value than the other samples.

Aroma compounds are volatile; they are perceived primarily with the nose. Sample C received the highest rating in terms of aroma**.** The results of the analysis showed that the treatment had a significant effect on overall acceptability of the watermelon juice in terms of the rating. Sample C also received the highest rating in terms of general acceptability. It was significantly different from sample E and similar to samples A, B, D and the control sample. The findings of this study showed that the extract of serendipity berry performed the dual role, that of a sweetener and a preservative.

Table 2. Microbial count of the juice samples at the end of the first week

SAMPLE CODE	BACTERIAL COUNT		MOULD COUNT
	NA (×10^5CFU/ml)	MA (×10^5 CFU/ml)	PDA (×10^3 CFU/ml)
A	0.3	0.4	0.4
B	0.8	<10.0 CFU/ml	1.2
C	<3.0 CFU/ml	<5.0 CFU/ml	<10.0 CFU/ml
D	<5.0 CFU/ml	<3.0 CFU/ml	<10.0 CFU/ml
E	<10.0 CFU/ml	NG CFU/ml	0.6
CONTROL	1.1	2.0	0.7

Legend: *SAMPLE A- 100 ml Watermelon + 10 ml of serendipity berry; SAMPLE B- 100 ml Watermelon + 20 ml of serendipity berry; SAMPLE C- 100 ml Watermelon + 30 ml of serendipity berry; SAMPLE D- 100 ml Watermelon + 40 ml of serendipity berry; SAMPLE E- 100 ml Watermelon + 50 ml of serendipity berry; CTR- Watermelon Juice only (Control)*

Table 3. Microbial count of the juice samples at the end of the sixth week

SAMPLE CODE	BACTERIA COUNT		MOULD COUNT
	NA (×10^6 FU/ml)	MA (×10^6 CFU/ml)	PDA (×10^3 CFU/ml)
A	0.2	0.3	1.3
B	<8.0 CFU/ml	0.4	1.2
C	<10.0 CFU/ml	1.0	<5.0 CFU/ml
D	<10.0 CFU/ml	<5.0 CFU/ml	<10.0 CFU/ml
E	<10.0 CFU/ml	<10.0 CFU/ml	1.0
CONTROL	4.5	5.1	4.3

Legend: *SAMPLE A- 100 ml Watermelon + 10 ml of serendipity berry; SAMPLE B- 100 ml Watermelon + 20 ml of serendipity berry; SAMPLE C- 100 ml Watermelon + 30 ml of serendipity berry; SAMPLE D- 100 ml Watermelon + 40 ml of serendipity berry; SAMPLE E- 100 ml Watermelon + 50 ml of serendipity berry; CTR- Watermelon Juice only (Control)*

Table 4. Microbial count of the juice samples at the end of the twelfth week

SAMPLE CODE	BACTERIA COUNT		MOULD COUNT
	NA ($\times 10^5$ CFU/ml)	MA (CFU/ml)	PDA ($\times 10^3$ CFU/ml)
A	0.4	1.4×10^5	1.2
B	1.1	1.0×10^3	1.3
C	<10.0 CFU/ml	0.3×10^3	1.3
D	<10.0 CFU/ml	0.2×10^3	1.0
E	NG	0.1×10^5	1.0
CONTROL	$9.2x10^7$	7.1×10^5	3.3

Legend: *SAMPLE A- 100 ml Watermelon + 10 ml of serendipity berry; SAMPLE B- 100 ml Watermelon + 20 ml of serendipity berry; SAMPLE C- 100 ml Watermelon + 30 ml of serendipity berry; SAMPLE D- 100 ml Watermelon + 40 ml of serendipity berry; SAMPLE E- 100 ml Watermelon + 50 ml of serendipity berry; CTR- Watermelon Juice only (Control)*

Table 5. Sensory evaluation of the juice samples

SAMPLES	COLOUR	TASTE	AROMA	GENERAL ACCEPTABILITY
Sample A	6.60 ± 0.50^{ab}	6.00 ± 0.46^{b}	6.07 ± 0.03^{b}	6.67 ± 0.45^{ab}
Sample B	7.00 ± 0.13^{ab}	7.07 ± 0.30^{ab}	6.93 ± 0.34^{ab}	7.07 ± 0.22^{ab}
Sample C	7.40 ± 0.35^{ab}	7.40 ± 0.44^{a}	7.33 ± 0.11^{a}	7.60 ± 0.12^{a}
Sample D	7.53 ± 0.83^{a}	6.87 ± 0.69^{ab}	6.53 ± 0.73^{ab}	7.13 ± 0.25^{ab}
Sample E	6.47 ± 0.64^{b}	5.93 ± 0.71^{b}	6.13 ± 0.81^{b}	6.20 ± 0.66^{b}
Control	6.95 ± 0.28^{ab}	6.81 ± 0.29^{ab}	6.38 ± 0.24^{ab}	6.67 ± 0.07^{ab}

Means with different letters in each column are significantly different ($P < 0.05$). Legend: SAMPLE A- 100 ml Watermelon + 10 ml of serendipity berry; SAMPLE B- 100 ml Watermelon + 20 ml of serendipity berry; SAMPLE C- 100 ml Watermelon + 30 ml of serendipity berry; SAMPLE D- 100 ml Watermelon + 40 ml of serendipity berry; SAMPLE E- 100 ml Watermelon + 50 ml of serendipity berry; CTR- Watermelon Juice only (Control)

Conclusions

The study was able to establish the fact that the extract of a serendipity berry has a significant effect on the shelf life and sweetening properties of the treated juice samples. It could be concluded that watermelon juice blended/sweetened with 30ml of the serendipity berry extract was rated higher when compared to other samples. The addition of a serendipity berry to the watermelon juice assisted in extending the shelf life of the juice for twelve weeks, thus making it an available meal ready to serve, refreshing drink with a good nutritional, medicinal and caloric value. The results of this research work affirmed both the susceptibility of pure watermelon to a microbial attack due to its high moisture content, and the preservative potentials of the serendipity berry extract. It was observed that the serendipity berry extract hadtwo major roles in the juice samples; that of a sweetener (natural) and a preservative.

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