Effects of heat, light and pH on the stability of pigments produced by *Talaromyces purpurogenus*

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Introduction

Natural colourants are used in foods, textile, pharmaceutical and cosmetics

They are preferred to synthetic ones since they are safer for human health and the environments.

Production of microbial pigments have continued to dominate other natural sources such as higher plants and animals because of the fast growth rate and diverse compositions and colours of pigments produced by these lower organisms. Filamentous fungi have been reported by many authors as the leading group in the production of natural colourants and dyes.

One of the major problems with natural pigments is their un-stability during processing and storage.

Objectives

- **The aim of this work was to study the effects of exposure to heat, light and pH on the colour stability of ethyl acetate extract of the pigments produced by *Talaromyces purpurogenus***

Methodology

Talaromyces purpurogenus **LC128689 was isolated from soil sample collected from cassava processing site in Abakaliki , Ebonyi State, Nigeria. The fungus was identified based on the gene sequence of the ITS region of ribosomal DNA (Ogbonna *et al*., 2017). The fungus was maintained on PDA slant at 4ºC and sub-cultured once in every six weeks. All the media components used in this experiment were procured from Wako Pure Chemical Industries ltd, Japan unless otherwise stated.**

Activation of the fungus

The fungus was activated by sub-culturing into potato dextrose agar (39g/L) test tube slants and incubating at room temperature (25±3°C) for 7 days. The fully sporulated test tube slants were stored in a refrigerator at 4°C for the experiments.

Pigment production in submerged shake flask cultures

The medium was composed of the following (g/L) : sodium nitrate, 0.8; magnesium sulphate, 0.4; peptone, 15.2; and glucose 25. The culture medium was dispensed in 100 ml aliquots into 500 ml Erlenmeyer flasks and sterilized by autoclaving at 121°C for 15 min. Sterilized distilled water was used to harvested *T. purpurogenus* spores from 7 days old PDA test tube slants and diluted to a concentration of $8.32x10^7$/ml (Ogbonna *et al.*, 2017). After cooling, each flask was inoculated with 4 ml of spore suspension containing the above spore concentration and cultivated at 30°C and 200 rpm for 7 days.


PYREX
250

Extraction of the ethyl acetate soluble components of the pigment

The culture broth was filtered through sodium acetate membrane filter and centrifuged at 1500rpm for 10 minutes. The supernatant was used for ethyl acetate extraction. The clean red coloured culture broth 350 ml was poured into a separation funnel and 30 ml of ethyl acetate was added. The mixture was shaken vigorously to mix and allowed to stand for 30 minutes until the upper ethyl acetate soluble pigment was clearly separated from the insoluble lower aqueous layer. The lower aqueous layer was carefully emptied into a conical flask through the lower valve while the upper ethyl acetate soluble layer was collected into another clean conical flask. The same process was repeated 5 consecutive times until most of the ethyl acetate soluble components of the pigments were extracted.

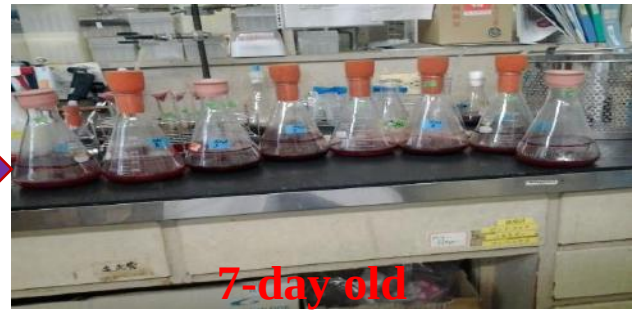
Drying the ethyl acetate extract with a rotary evaporator (Make and model)

A total of 150 ml of ethyl acetate soluble component was extracted from the 350 ml culture broth. The pigments soluble in ethyl acetate were recovered by evaporation in a rotary evaporator. The rotary evaporator was set at a temperature of 50^{o}C and pressure of 90mHg and the ethyl acetate was evaporated to dryness. The dry weight of the recovered pigments was 0.719g. The dry extract was stored overnight in a desicator impregnated with silica gel to avoid moisture absorption. The recovered dry extract was used in the stability experiments.

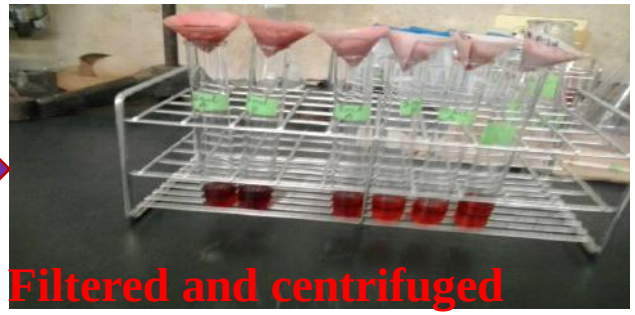
•Preparation of pigment extract for various treatments

•The dried ethyl acetate extract of pigment was weighed 3.060 mg into a 10 ml beaker and 5 ml of absolute ethanol was added to solubilize the extract. The solution was transferred into 10 L glass beaker and 7.455 ml of distilled water was added to give a final concentration of 0.408 mg/ml. The initial pH of the extract solution was 3.2. The initial colour intensity was determined by reading the optical density at 400, 460 and 500 nm for yellow, orange and red pigments respectively.

3-day old



7-day old



Filtered and centrifuged

Extracted with ethyl Acetate and separated

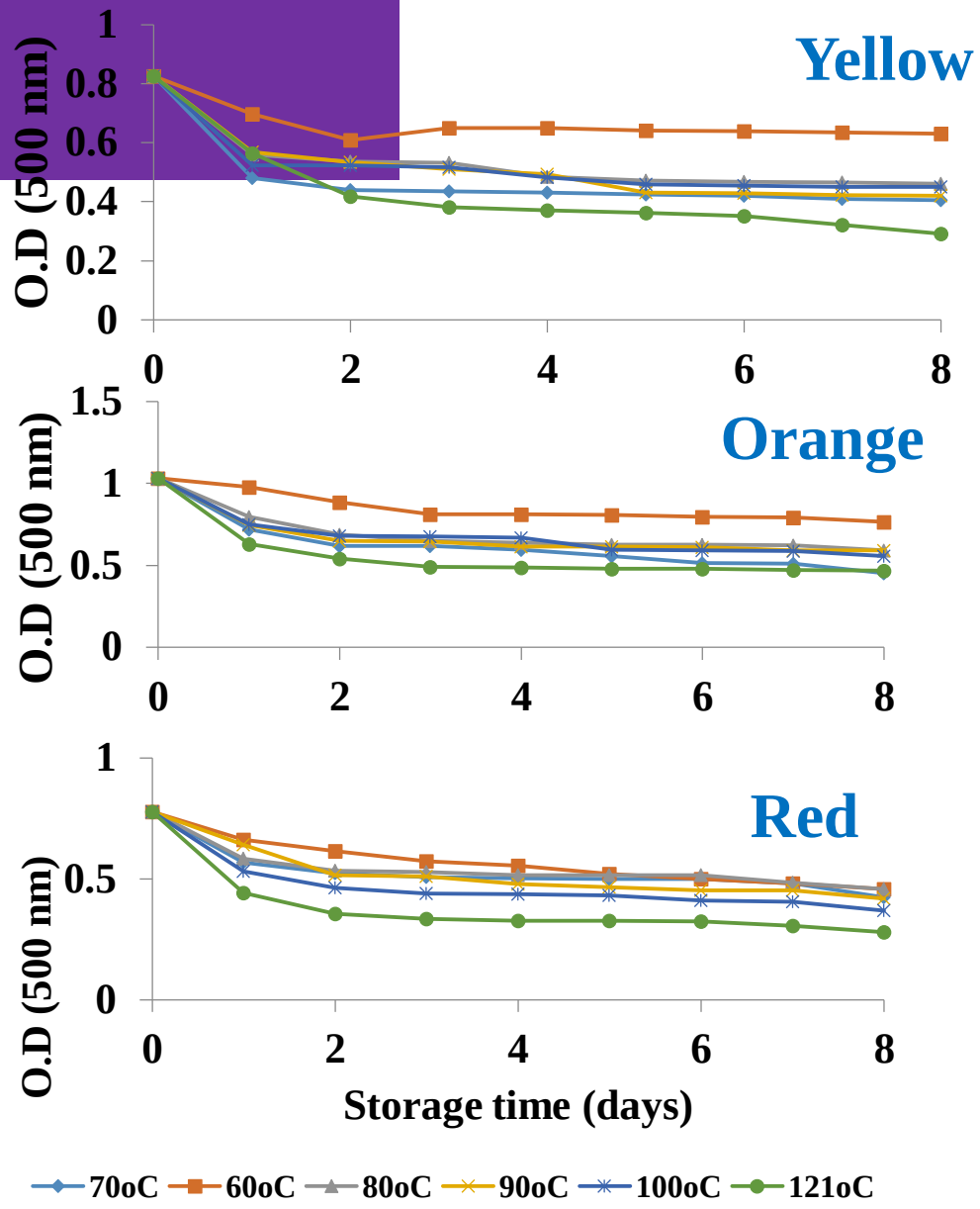
The extract is evaporated

The extract is evaporated

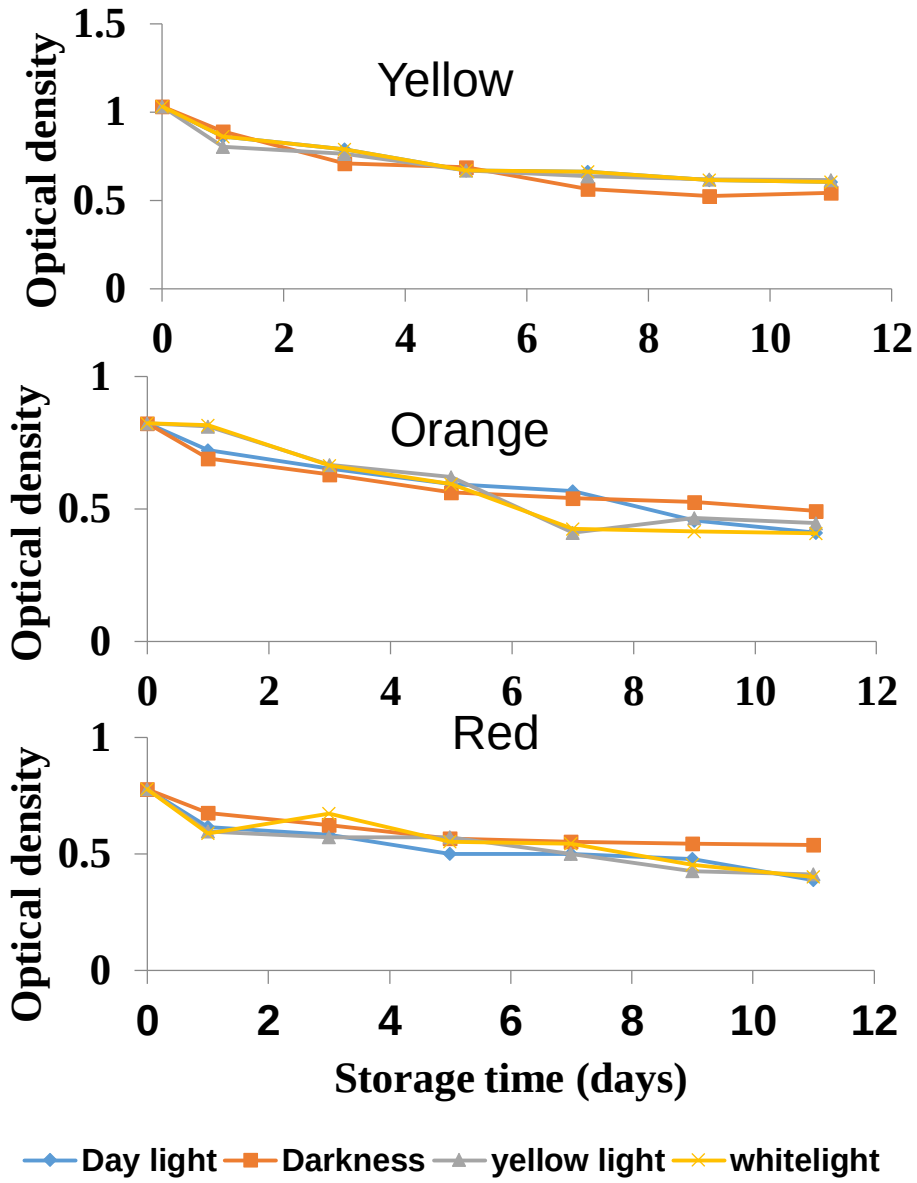
The recovered extract is dissolved in methanol, diluted with distilled water and used for stability studies

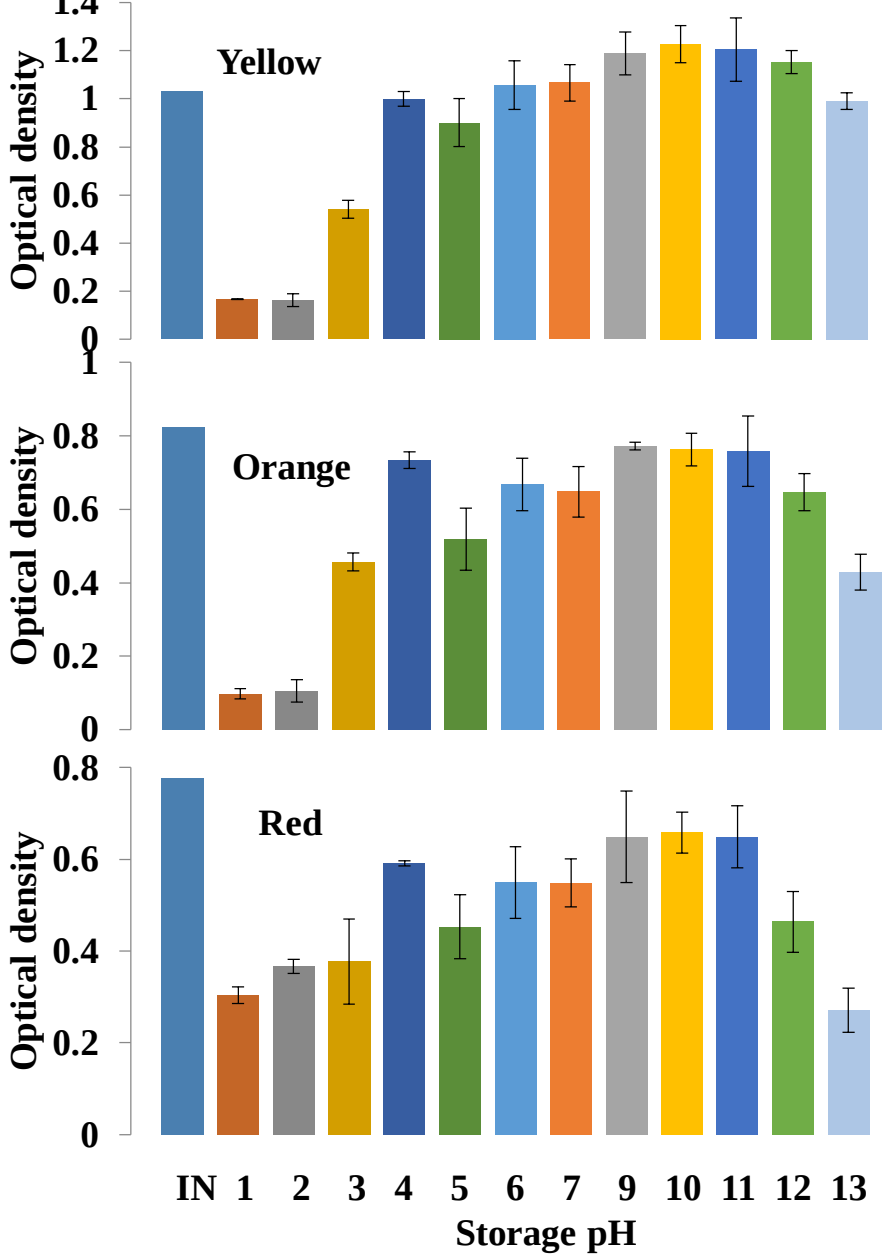
Effects of heat on colour stability of the ethyl acetate extract of *T.purpurogenus* pigments

The pigment solution was dispensed in 40 ml aliquots into eighteen (18) 100 ml brown reagent bottles. The bottles were grouped into six with three bottles per group. Each group was heated for 30 minutes at different temperatures: 60, 70, 80, 90 and 100^{o}C respectively in a water bath (make) and at 121^{o}C in an autoclave. After heat treatment and cooling each bottle was wrapped in aluminium foil and placed inside a dark wooden box to avoid light penetration. The temperature was maintained at 25 ±3^{o}C. Samples were withdrawn daily from each bottle to measure the pH and optical density of the pigment for a period of 21days.



Effects of heat treatment on stability of yellow、 orange and red pigments produced by *Talaromyces purpurogenus*.


Yellow
Orange
Red
Optical density
1.5
1
0.5
0
0
2
4
6
8
10
12
Storage time (days)
Day light
Darkness
yellow light
whitelight



Effects of pH on stability of yellow, orange and red pigments produced by *Talaromyces purpurogenus*.

Conclusions

The effects of various treatments on the stability of the three pigment colours produced by *T. purprogenus* vary. However, the colours are fairly stable and thus can be used for various applications.

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References

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