Journal of Food Engineering 164 (2015) 21–29

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Effect of heat exposure on the colour intensity of red pigments produced by *Penicillium purpurogenum* GH2

L. Morales-Oyervides [a], J.C. Oliveira [a,*], M.J. Sousa-Gallagher [a], A. Méndez-Zavala [b], J.C. Montañez [b]

[a] Department of Process and Chemical Engineering, University College Cork, Cork, Ireland
[b] Department of Chemical Engineering, Universidad Autónoma de Coahuila, Saltillo, Coahuila, Mexico

ARTICLE INFO

Article history:
Received 2 July 2013
Received in revised form 14 April 2015
Accepted 17 April 2015
Available online 25 April 2015

Keywords:
Kinetics
Mathematical modelling
Pasteurisation
Thermal processing

ABSTRACT

Natural pigments are alternatives to synthetic colorants with growing industrial interest for food and non-food uses (e.g. textiles). However, they are generally thermally labile and therefore their practical interest depends on the loss under typical processing conditions being acceptable. *Penicillium* strains are producers of natural red pigments of potential interest. The loss of colour of these pigments was studied at relevant conditions for food and beverage pasteurisation. Thermal liability of colour was well described by a first order model (with a constant off-set) at pH 6 up to 80 °C, and at 80 °C for pH 4–8, with a *z*-value of 48.7 ± 0.9 °C and a $D_{80C,pH6}$ of 981 ± 5 min. The stability was significantly affected by pH, with an approximately linear increase of 173 ± 3 min in D_{80} per pH unit. The high *z*-value, which is 5 times the *z* of typical target micro-organisms in thermal processing, suggests a good scope for process optimisation to minimise losses. At the middle point of pH 6 these kinetic parameters would suggest that losses of red colour intensity (*OD* at 500 nm) of less than 1% in pasteurisation are feasible. Losses will be higher in matrices of lower pH, where process optimisation will therefore be more relevant. It was concluded that these red pigments are of industrial interest, as with further optimisation of industrial production yields to minimise costs, and then going through the process of acceptance as new food ingredients, they are potentially economically competitive.

1. Introduction

Colour is one of the most important sensory attributes of foods, contributing to their acceptance by consumers. Several studies along with demographic observations showed the importance of colour as a single factor on which food preference is often based (Clydesdale, 1993). Recently, there has been a high interest in the utilisation of natural ingredients by the food industry since various synthetic pigments have been associated with toxic effects in foods (Mapari et al., 2005). It has been reported (Cho et al., 2002; Dufossé, 2006; Méndez-Zavala et al., 2011; Suhr et al., 2002) that some microorganisms have the ability to produce pigments in high quantities. Microorganisms are more feasible sources of pigments in comparison to pigments extracted from plants and animals because they do not have seasonal constraints, do not compete for limited farming land with actual foods, and can be produced in high yields (De Carvalho, 2004). *Monascus* pigments have been suggested as food colorants, but they contain citrinin, which therefore limits the likelihood of their approval (Mapari et al., 2008). It is therefore important to find non-toxic pigment microorganism producers other than *Monascus* as an alternative. Recently, *Penicillium purpurogenum* has gained attention as a pigment producer strain in liquid fermentation (Méndez-Zavala et al., 2011; Santos-Ebinuma et al., 2013) and furthermore *Penicillium* colorants have presented antimicrobial activities and absence of toxicity against *Artemia salina* (Teixeira et al., 2012).

The stability of natural pigments under processing conditions has been a major challenge for food applications (Rawson et al., 2011) because natural colorants tend to be thermally labile, which limits their industrial interest. The application of heat is the most common technology used in food processing and preservation. However, heat is known to induce several chemical and physical changes that may reduce the content or bioavailability of some bioactive and functional compounds (Patras et al., 2010; Rawson et al., 2011; Sánchez-Moreno et al., 2005). Thermal processing of foods involves heating to temperatures from 50 to 150 °C, depending on the pH of the product and the desired shelf life. The stability of natural pigments is therefore the main focus of many recent

* Corresponding author.
E-mail address: j.oliveira@ucc.ie (J.C. Oliveira).

http://dx.doi.org/10.1016/j.jfoodeng.2015.04.020
0260-8774/

studies due to their abundant potential applications, their beneficial effects and their use as alternatives to artificial colorants in foods (Mapari et al., 2010; Puspita et al., 2012).

These studies have shown that natural pigments may have too low stability for food processing and therefore it is necessary to ascertain if pigments have sufficient stability for practical use before expensive research is undertaken to have them accepted as new food ingredients, under the constraints of modern legislation. Pigment stability is not only a function of the processing temperature, it is also influenced by intrinsic properties such as pH, chemical structure, enzymes, protein and metallic ions content, and also extrinsic factors during storage, such as light and oxygen availability (Ahmad et al., 2012; Chiste et al., 2010; Jing et al., 2012; Mapari et al., 2009; Ranalli et al., 2005; Shi and Le Maguer, 2000; Wada and Kishikawa, 2007). There are many studies on the thermal degradation of food compounds such as anthocyanins, carotenes and enzymes (Aparicio-Ruiz et al., 2011; Hernández-Herrero and Frutos, 2011; Rudra-Shalini et al., 2008), but there is scarce information in literature on the kinetics of thermal degradation of natural pigments produced by microorganisms. Mapari et al. (2009) studied the photostability of fungal pigments produced by *Penicillium aculeatum* IBT 14263 and *Epicoccum nigrum* IBT 41028, concluding that fungal pigments presented higher photostability in comparison with the controls. Recently, Terra-Silveira et al. (2013) studied the thermal and pH degradation of red pigments produced by the fungus *Monascus purpureus*, concluding that a first-order kinetic model was suitable to describe the thermal degradation of pigments.

Red pigments are very important for many sauces (e.g. tomato, chilli) and some beverages (e.g. berries), so its most relevant applicability would be in aqueous solution. In order for this process to have practical application, it is necessary to establish that (i) it is sufficiently stable and amenable to process optimisation to minimise losses under conditions of interest to foods and drinks; (ii) it can be produced industrially with high yields; (iii) it is characterised and tested in accordance with EU and FDA regulations for use as a new food ingredient. This sequence of steps is listed from cheapest to most expensive, as there is no point in engaging in more expensive research if the economic feasibility of the applications is not assured.

The objective of this work was to quantify the kinetics of the thermal degradation of the red pigment extract produced by fermentation of *P. purpurogenum* GH2 at relevant conditions for pasteurisation of foods and beverages, in order to assess the extent of its loss, and the potential to minimise it to acceptable levels with process optimisation.

Thermal processing optimisation is based on achieving the pasteurisation target (lethality of target microorganisms) while minimising the loss of the valuable components. Due to the different sensitivity to temperature of microbial lethality and most quality factors, HTST (high temperature short time) conditions are ideal (Awuah et al., 2007). Thermal processing is most commonly defined on the basis of the Bigelow Thermal Death Time (TDT) kinetic model, where temperature influence is quantified by the *z*-value, which is of the order of 8–12 °C, depending on product and microbial target. The higher the differential of the *z*-value of the quality factor to this target, the greater the loss minimisation achieved by HTST. Thus, the red pigment extract studied in this work would be of industrial interest if it showed a high *D* value and a high *z* value.

It could be visibly seen that loss of pigment resulted in loss of colour intensity as well as light absorbance at this wavelength. Thus, the Optical Density at 500 nm (OD_{500}) is a measure of both the pigment concentration and of the colour intensity sensorial perceived.

2. Materials and methods

2.1. Microorganism

P. purpurogenum GH2 was used for pigment production. The purified strain was donated by DIA-UAdeC (Cruz-Hernández et al., 2001; Espinoza-Hernández, 2004). Cultures of this microorganism were maintained on PDA (Potato dextrose agar, 39.0 g L^{-1}) slants at 4 °C and sub-cultured periodically.

2.2. Media

The Potato Dextrose Broth (ATCC medium: 336) was prepared by boiling finely 300.0 g of diced potatoes in 500 mL of water until thoroughly cooked; the potatoes were then filtered through cheese-cloth and water was added to the filtrate to complete a volume of 1.0 L. The filtrate was then heated to dissolve the agar. Finally, 20.0 g of glucose was added before sterilization. The culture growth medium used was the Czapek-dox modified medium reported by Méndez-Zavala (2011) for the production of pigments, consisting in (g L^{-1}): *D*-xylose 15.0, $NaNO_3$ 3.0, $MgSO_4{\cdot}7H_2O$ 0.5, $FeSO_4{\cdot}$ $7H_2O$ 0.1, K_2HPO_4 1.0, KCl 1.0 and ethanol 20.0.

2.3. Inoculum preparation

Erlenmeyer flasks (125 mL)containing 25 mL of PDB medium were sterilized and inoculated with a spore suspension (1×10^5 spores mL^{-1}) of *P. purpurogenum* GH2. They were cultivated at 30 °C for 3 days in an orbital shaker (Inova 94, New Brunswick Scientific, USA) at 180 rpm.

2.4. Culture conditions for pigment production

The initial pH of the Czapek-dox modified medium was adjusted to 5.0 before sterilizing by using 0.22 μm sterile membranes (Millipore, USA). A mycelial suspension of *P. purpurogenum* GH2 was inoculated at 10% (v/v) in 125 mL Erlenmeyer flasks containing 25 mL of medium. The inoculated flasks were incubated at 30 ± 2 °C in an orbital shaker (Inova 94, New Brunswick Scientific, USA) at 200 rpm for 6 days.

2.5. Pigment extraction

The pigment extraction was performed according to the methodology reported by Méndez-Zavala (2005). The pigment extract was centrifuged at 8000 rpm and at 4 °C for 20 min (Sorball, Primo R Biofuge Centrifugation Thermo, USA). The supernatant was then subjected to a second centrifugation at 8000 rpm for 30 min and at 4 °C, and finally filtered through a 0.45 μm cellulose membranes (Millipore, USA) for the subsequent analysis of pigments. In this study only extracellular pigments were considered. The analysis of red pigment production was done by measuring the absorbance of the filtered extract at 500 nm using a spectrophotometer (Cary 50, UV–Visible Varian, USA) taking the dilution factor of the samples into consideration. This wavelength was selected by scanning the maximum sensitivity for the presence of the pigment (that is, the pigment absorbs maximum light at this wavelength).

2.6. Mathematical modelling of colour loss kinetics

The most common kinetic model to quantify microbial lethality is Bigelow's Thermal Death Time (TDT) model, which assumes a logarithmic decrease at constant temperature with the *D*-value