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Analytical Methods

Development of an optimised papaya pulp nectar using a combination of irradiation and mild heat

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ABSTRACT

Papaya does not sufficiently maintain desired fresh fruit quality when shipped long distances due to an easily bruised soft skin and a short shelf life. This leads to both a large supply of pulp from unsightly fruit that is never shipped and low sales due to blemished fruit. Unfortunately, traditional preservation methods (pasteurisation) negatively alter papaya's fresh flavour. Thus to effectively utilise available papaya pulp, processing requires an approach that enables retention of papaya's natural flavours without excessive heat. Papaya fruit (*Carica papaya* L., var.'s *Rainbow* (yellow-fleshed) and *SunUp* (red-fleshed)) were pulped, diluted, and processed with mild heat (80 °C, 5 min), irradiation (5 kGy or 7.5 kGy) or combinations of both. Irradiation resulted in a significant reduction in ascorbic acid content. Mild heat treatment significantly reduced pectinesterase activity and microbiological viability. Irradiation followed by heat further enhanced destruction of *Listeria innocua* and *Clostridium sporogenes* and retained the flavour and a nutritional profile closest to untreated controls. The product was microbiologically safe with acceptable enzyme levels and would be shippable under refrigeration.

1. Introduction

The papaya plant (*Carica papaya* L.) is grown in every tropical and subtropical country. A tree-like herbaceous crop, it is a member of the Caricaceae family. The flavour is like a cantaloupe; it is sweet and juicy, though with some muskiness (Morton, 1987).

Papaya ranks highest per serving among fruit for carotenoids, potassium, fibre, and ascorbic acid content (Liebman, 1992; USDA National Nutrient Database for Standard Reference). Papaya contains 108 mg ascorbic acid per 100 g of fresh fruit, which is higher than oranges (67 mg/100 g, Lim, Lim, and Tee (2007)), and has the highest flavonoid content (*v. exotica*) amongst all other Mauritian exotic fruits examined by Luximon-Ramma, Bahorun, and Crozier (2003) except guava. Among a large sample of Mediterranean and tropical fruits, papaya fruit ranked second only to *Passiflora eduliss* var. *Eduliss* for its hydroxyl radical and hydrogen peroxide scavenging activity (Murcia, Jimenez, & Martinez-Tome, 2001). Unfortunately, these excellent nutritional qualities are only available where the fresh fruit is available.

The papaya industry capacity is underutilised. First, fresh whole papaya fruit does not transport well; bruising due to a soft skin, a short shelf life, and potential chill injury do not allow for (or make very costly) shipments from Hawaii to travel beyond the west coast of the continental US (Hawaiian Papaya Industry Association, personal communication) and maintain the desired quality. Papaya available in the Midwest is typically shipped from South America, but suffers from the same problems (evaluation of grocery store chain suppliers in Champaign, IL). Fruit is often soft, wrinkled and/or bruised, significantly decreasing consumer acceptance in an environment where unblemished fruit is expected. Second, a processed papaya product that maintains its fresh flavour does not currently exist. Research on processing of papaya fruit has resulted in a sweet (non-bitter), stable product; traditional pasteurisation treatment leads to cooked flavour development (Argaiz & Lopez-Malo, 1995). Other heat processed fruits, including apples, oranges and tomatoes, do not suffer such an extreme alteration from their fresh fruit flavour after processing. This has led to papaya products that are mixed with other fruits to dilute or mask the off-flavours (Tiwari, 2000). The overall result is a stunted papaya industry due to the detrimental effects of traditional heat processing on papaya flavour and aroma. Therefore, papaya requires an approach which would allow retention of more of its

Abbreviations: C, control; H, heat; 5, 5 kGy; 7.5, 7.5 kGy; 5H, 5 kGy + heat; 7.5H, 7.5 kGy + heat; CFU, colony forming unit; EPR, electron paramagnetic resonance; HPLC, high performance liquid chromatography; ORAC, oxygen radical absorbance capacity; RT, room temperature.

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natural flavours, while maintaining inhibition of bacterial and enzymatic degradation. Mild heat treatment would be sufficient enough to destroy vegetative microorganisms, removing that requirement from the irradiation process, without strongly impacting flavour. The application of irradiation was hypothesised to offer other benefits, such as improved texture, additional protection from spore-forming organisms, and better retained nutrients.

Minimal literature reports exist on irradiation of juices. Papaya juice has been shown to quench hydroxyl free radicals formed during exposure to gamma rays (Gray & Mower, 1991). Glucose, fructose and sucrose were found to primarily absorb the rays, with malonaldehyde and other sugar derivatives being formed. Niemand, den Drijver, Pretorius, Holzapfel, and van der Linde (1983) reported that irradiation of sugar solutions resulted in mutagenic compounds; these mutagenic compounds did not form in irradiated mango juice. Organic acids and phenols contributed to 85% of the protective effect. These results would likely apply to papaya as well, since the sugar, organic acid and phenol contents are similar (Luximon-Ramma et al., 2003). A WHO Technical Report (1997) also concluded there were no safety concerns with high (>10 kGy) doses of irradiation.

In designing a process for preparing papaya pulp, pasteurisation was first explored (Brekke, Chan, & Cavaletto, 1973). However, cooked flavour development in papaya occurs to a much greater degree than in other pasteurised fruit products (Argaiz & Lopez-Malo, 1995). As a result, products have been developed that mix papaya with other components in an effort to minimise off-flavours produced from papaya heat treatment (Mostafa, Abd El Hady, & Askar, 1997; Tiwari, 2000). Producing a papaya beverage where the primary flavours come from papaya requires different processing approaches to avoid off-flavours produced by heat treatment. A combination of moderate irradiation and mild heat should decrease the need for flavour masking, and allow a higher percentage of the flavour to come from papaya.

The purpose of this research was to evaluate irradiation and mild heat treatment together as a viable alternative to heat treatment alone. Also, the aim was to minimise required processing as much as possible to maximise nutrient retention. It was hypothesised that a combination treatment using both irradiation and mild heat would result in a safe product that retained flavour and a nutritional profile more similar to fresh papaya than pasteurised pulp. This would make possible a wider acceptability of a fruit pulp with superior nutrient density.

2. Materials and methods

2.1. Materials

Carica papaya L. var.'s *Rainbow* and *SunUp* were donated by the Hawaiian Papaya Industry Association and the Hawaii Department of Agriculture. They were harvested when approximately 25% yellow and vapour-heat treated to eliminate fruit fly infestation (by heating to 47 °C over at least 4 h at 90% humidity, then cooling for 1 h) in Hawaii before shipment (second day air). Fruit were stored at 23 °C for 3–5 days until at least 80% yellow before pulping.

Escherichia coli K12 was provided by Dr. Scott Martin from the University of Illinois and cultured in Luria-Bertani (LB) broth. *Listeria innocua* (ATCC, Manassas, VA) was received freeze-dried and cultured in Brain Heart Infusion medium (Fisher Scientific, Fairlawn, NJ). *Salmonella typhimurium* (Carolina Biological Supply, Burlington, NC) was received in MicroKwik nutrient agar and cultured in LB broth. *Clostridium sporogenes* (Carolina Biological Supply) was received in thioglycollate medium in a MicroKwik vial and cultured using reinforced Clostridium medium (Fisher Scientific). AAPH (2,2′-azobis(2-amidino-propane)dihydrochloride) was obtained from Wako Chemical (Richmond, VA). Trolox, ascorbic acid, ferrous sulphate and EDTA were purchased from Fisher Scientific. Fluorescein, α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN), quercetin and pectin were acquired from Sigma Chemical (St. Louis, MO).

2.2. Sample preparation

Papaya was divided into sets (5 fruit per set) which were washed, dried, halved, and the seeds were removed. The pulp was transferred into a KitchenAid K5SS with a pulper attachment (Hobart Corporation, Troy, OH) and collected. Brix was measured using a Westover Scientific (Mill Creek, WA) RHB-32ATC hand held refractometer and papaya pulp was diluted with double distilled H_2O (ddH_2O) to 8 °Brix. Diluted pulp was separated into glass containers labelled for the following treatments and frozen (−20 °C): control (C), heated to 80 °C for 5 min (H), 5 kGy irradiation (5), 7.5 kGy (7.5), 5 kGy + heat (5H), 7.5 kGy + heat (7.5H). Samples were irradiated in triplicate while frozen in a Gammacell 220 Excel with a cobalt-60 source (MDS Nordion, Ottawa, ON, Canada). Combination treatments were irradiated prior to heat treatment.

2.3. Titratable acidity

Titratable acidity was performed according to a modified AOAC (2002, chapter 11) method, conducted as follows: the pH of 5 g diluted papaya was measured after addition of 125 ml ddH_2O. This solution was quickly titrated with 0.02 N NaOH to reach a final pH 8.2. The volume of NaOH consumed was used to calculate citric acid equivalents.

2.4. Viscometry

Freshly thawed papaya samples were centrifuged (to remove pulp) and transferred onto the Advanced Rheometric Expansion System Rheometric Fluid Spectrometer III (ARES RFS III) with an attached SR5 Peltier Circulator (TA Instruments, New Castle, DE). It was connected to TA Orchestrator Software Version 8.03 (TA Instruments) with the gap set at 0.5 mm and a temperature of 5 °C. Treatment samples were run in triplicate and ddH_2O was used as a standard for calibration. Values are reported as shear rate/shear stress (s^{-1} Pa^{-1}).

2.5. Colourimetry

Papaya samples were poured into a glass container, placed on the reading area of the HunterLab LabScan II 0/45 (Hunter Associates Laboratory Inc., Reston, VA), and covered. Measurements were analysed with HunterLab Universal Software™ Version 3.8. After automatic standardisation, samples were analysed in triplicate. Each treatment was compared to the control with respect to differences in L, a, b, and ΔE values.

2.6. Microbiology

Four non-pathogenic or opportunistic organisms were selected to represent a variety of potential contaminating organisms (Busta et al., 2003). Frozen *E. coli* K12 cultures were thawed and added to LB media. Cultures were incubated (24 h, 37 °C) with rotation. Thawed *Rainbow* and *SunUp* papaya pulp samples were spiked with sufficient *E. coli* K12 culture to result in 10^{10} CFU of *E. coli* added per container. Inoculated papaya pulp was frozen overnight and treated the following day, after which inoculated papaya was plated on LB plates in duplicate. The plates were then incubated (24 h, 37 °C). Observations for *S. typhimurium* were conducted in