Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 September 2020 doi:10.20944/preprints202009.0232.v1

Technical and Economic Feasibility of a Stable Yellow Natural Colorant Production from Waste Lemon Peel

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Abstract: A brief technical and economic insight into producing the water-soluble yellow colorant limocitrol 3-*O*-6"-[3-hydroxyl-3-methylglutaryl)])-β-D-glucopyranoside from waste lemon peel via simple solid-liquid extraction in aqueous ethanol or via hydrodynamic cavitation of waste lemon peel in water, shows that the biocolorant can be obtained at affordable cost. Coupled to the simplicity and sustainability of the extraction processes suggested, the high chemical and physical stability of this polymethoxylated flavanol and the health benefits of citrus flavonoids, support industrialization of this new bioeconomy production.

Keywords: limocitrol; lemon; citrus; bioeconomy; biocolorant; flavonoids

1. Introduction

Following the 2007 study linking hyperactivity in 300 children to consumption of foodstuff and beverages using synthetic azo-dyes along with preservative sodium benzoate [1], the six azo-dyes synthetically derived from tar (sunset yellow, quinoline yellow, carmoisine, allura red, tartrazine, ponceau 4R) widely employed as pigment food additives are increasingly replaced by naturally derived colorants [2].

Water-soluble sunset yellow (E110 in the European labeling for food additives, Yellow 6 in the USA), quinoline yellow (E104, Yellow 10 in the USA) and tartrazine (E102, Yellow 5 in the USA) water-soluble "aza" dyes can be replaced by combinations of natural colorants such as curcumin, paprika, annatto, lutein, carotene and crocin.

For example, the 2011 guidelines to the replacement of artificial dyes commissioned to industry by the Food Standards Agency in Scotland highlighted that "smoked haddock used to be colored with either tartrazine or quinoline yellow, but these are now combination of annatto and curcumin, while another company uses curcumin and paprika in its haddock fillets" [3].

The need to access natural yellow dyes of higher stability, lower cost and abundant supply is widespread, especially considering that tartrazine is not only used in foods, but also in personal care, cosmetic and medication products [4], and that carcinogenic contaminants in Yellow 5 such as benzidine, whose limit in the USA is 1 part per billion, in certain dye batches was found to actually amount to 83 ppb (free and bound benzidine) [5].

The main problems with biocolorants derived from natural sources are the lower chemical and physical stability and higher cost (2-10 fold higher [2]) compared to synthetic counterparts.

Chemical technologies such as microencapsulation or dye stabilization with naturally derived polyphenols can be used to develop biocolorant formulations whose stability is comparable to that of artificial dyes. In the field of red colorants, examples include lycopene micronized crystals formulated in glycerol [6] and betanin in water from *Opuntia ficus-indica* peel stabilized by the fruit peel biophenols [7].

In 2019 Hamann and co-workers identified and isolated limocitrol 3-*O*-6"-[3-hydroxyl-3-methylglutaryl)])-β-D-glucopyranoside, namely a flavanol glycoside called Yellow 15, in the hydroalcoholic (ethanol plus water) extract of the zest of *Citrus limon* fruits [8].

In this study we investigate the technical and economic feasibility of Yellow 15 production from waste lemon peel, namely a by-product of the citrus industry widely employed as raw material to manufacture highly valued pectin [9] and lemon essential oil in the context of the rapidly unfolding lemon bioeconomy [10].

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2. Technical and economic aspects

The procedure to extract limocitrol 3-*O*-6"-[3-hydroxyl-3-methylglutaryl)])-β-D-glucopyranoside from the zest of *Citrus limon* includes three extraction steps of lemon zest with 95% water and 5% ethanol, followed by another three extraction steps with 50%:50% water:ethanol mixture (Figure 1) [8].

Figure 1. Ground lemon zest is extracted Extract three times (1,2,3 *left*) with 95:5 H_2O:EtOH followed by three new extraction rounds (4,5,6, *right*) with 50:50 H_2O:EtOH. [Picture by Professor Mark Hamann, The Medical University of South Carolina].

Eventually, Yellow 15 (5 g) is isolated via chromatography on Sephadex LH-20 column and HPLC with an overall yield of 1.4%. The dye easily dissolves in water from which it can be isolated via simple freeze-drying as a yellow powder (Figure 2).

Figure 2. Lemon-derived Yellow 15 in solution and in powder form [Pictures by Dr. Xiaoyan Chen and Prof. Mark Hamann, The Medical University of South Carolina].

The colorant is the limocitrol glycoside esterified with 3-hydroxymethylglutaric acid (Figure 3). This explains why it is considerably more soluble in water than curcumin, and more soluble of limocitrol-3-*O*-β-D-glucopyranoside, the glycoside of the lemon flavonol limocitrol first isolated in the form of yellow crystals in 1964 [11]. Indeed, after one month exposure to sunshine of equimolar solutions of Yellow 15, crocin and curcumin, significant color degradation of crocin and curcumin was observed, whereas color of the limocitrol-based dye showed little or no color degradation [8].

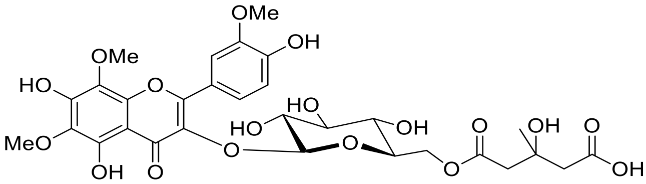
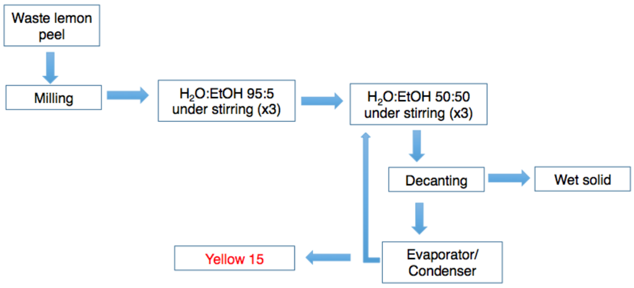


Figure 3. Chemical structure of Yellow 15: limocitrol 3-O-6''-(3-hydroxy-3-methylglutaryl)-β-D-glucopyranoside.

Displayed in Scheme 1, the hydroalcoholic extraction involves a first extraction milled (with an electric blender) waste lemon peel with EtOH (5% v/v, ×3) followed by a subsequent extraction with EtOH (50% v/v, ×3), in each case at room temperature. Following decanting, the biosolvent is recovered by evaporation under reduced pressure and reused in subsequent extraction runs.



Scheme 1. The extraction of Yellow 15 from waste lemon peel and recovery of ethanol used as co-solvent.

The extraction of the dye with aqueous ethanol from waste lemon peel at room temperature offers numerous advantages. First, ethanol is a common by-product of most citrus companies producing orange, lemon or grapefruit juice. For example, EtOH 91% (v/v) is commonly obtained following fermentation of the sugar-rich pulp residues of the centrifuge originating both from citrus essential oil and juice residues and also from the depulped and other residues of citrus squeezing [12].

Second, aqueous bioethanol is an eminently green extraction [13] meeting several of the principles of Green Extraction, including the first and second principles (use of renewable solvent) due to the agricultural origin of the solvent [14].

Third, the high solubility of this lemon flavonol glycoside in water makes its isolation a straightforward process using water, the cleanest possible solvent. Indeed, rather than separating the zest from the lemon peel, the industrial process could easily rely on the hydrodynamic cavitation (HC) of waste lemon peel in water.

Recently demonstrated on semi-industrial scale [15], the process enables to extract all valued water-soluble components of fresh (wet) waste lemon peel in one pot with high energy efficiency using no acid, base or organic solvent. The bioflavonoids imparting to lemon peel its yellow color are dissolved in water (Figure 4) from which can be easily isolated using an optimized version of the chromatographic process demonstrated on laboratory scale with the dye extracted with aqueous ethanol [8].

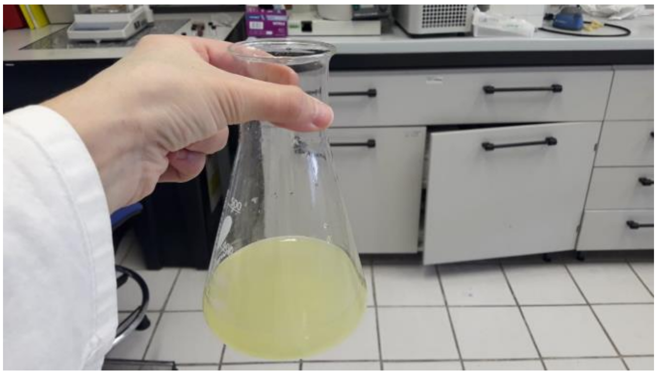


Figure 4. The yellow aqueous solution following hydrodynamic cavitation of waste lemon peel [Image courtesy of Dr Mario Pagliaro, CNR].

It also of fundamental importance, in light of forthcoming practical application, that the citrus bioproducts obtained via HC are devoid of microbial contamination due to the exceptional antibacterial activity exerted by the microbubble implosion taking place during cavitation [16], since dyes from natural sources are often contaminated by spoilage organisms and pathogens [17].

The two major costs faced by a company willing to extract this lemon-based colorant from waste lemon peel according to the solid-liquid extraction route based on use of hydroalcoholic solution of via HC in water only would be *i*) labor, and *ii*) electricity needed either to recover the bio-based solvent or to power the HC reactor. The latter cost can be easily and significantly reduced by self-producing power through a rooftop photovoltaic (PV) plant whose cost has become so low to make its installation affordable to any company in virtually all world's countries [18].

The cost of the raw material (waste lemon peel) available as by-product of lemon juice (and lemon oil) manufacturing plants is low whereas -- driven by powerful megatrend demand for healthy "naturals" in food, beverage, cosmetic, personal care and nutraceutical products -- demand and production of lemons in the last two decades has been growing steadily, with main producing countries expected to report increase in production by over 1.2 million tonnes in next five years (>700,000 tonnes in the northern hemisphere and > 550.000 tonnes in the southern hemisphere) [19].

3. Conclusions

Waste lemon peel obtained in about 50% in weight at lemon juice processing companies is an ideally suited raw material to produce, beyond valued pectin [9,15] and lemon oil, the water-soluble and highly stable yellow natural colorant limocitrol glycoside 3-*O*-6"-[3-hydroxyl-3-methylglutaryl)]-β-D-glucopyranoside. The dye is a bioflavonoid suitable as industrial alternative to both synthetic and water-soluble natural yellow colorants such as curcumin, crocin, norbixin or riboflavin (vitamin B2) [20].

It is also relevant here to learn that between 1980 and 2016, the lemon yearly share of the citrus market increased from 5% to 13%, going from 5.2 to 17.5 million tonnes [19], whereas entrepreneurial efforts (undertaken for example in the Netherlands [21]) to have waste citrus peel recognized from a regulatory viewpoint as a raw material and no longer as waste have been successful.

Extraction may be conducted via simple solid-liquid extraction in aqueous ethanol or via hydrodynamic cavitation of waste lemon peel in water, in both cases at affordable cost essentially dictated by the cost of labor and electricity.

In case of hydroalcoholic extraction, ethanol can be can be easily recovered by evaporation under reduced pressure [8], whereas in the hydrodynamic cavitation-based process no organic solvent is required to extract all valued water-soluble bioproducts [19].

Coupled to the ease and greenness of the extraction processes suggested, the enhanced stability of the natural colorant and the health benefits of citrus flavonoids (cardioprotective, chemopreventive, and neuroprotective effects) [22], support industrialization of this new bioeconomy production.

In the logics of the circular economy applied to citrus, resulting in what Siles López, Li and Thompson in 2010 called "the citrus biorefinery" [23], production of the yellow biocolorant would talk place concomitantly to production of all valued bioproducts obtainable from waste lemon peel, namely pectin, essential oil, cellulose and other flavonoids.

It is therefore important to develop new scalable green extraction technologies capable to afford all the aforementioned bioproducts, if possible in one pot and with high energy efficiency.

Once said biobased production will be industrialized, most likely directly at large citrus processing plants, this polymethoxylated flavanol derived from lemon made available both as standardized aqueous solution and in powdered form will be widely used in different beverage and food products including sports drinks, dairy and bakery products, fats, ice creams, dried fruits and cereals, as well as in cosmetic and personal care products still using hazardous synthetic yellow dyes.

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 doi:10.20944/preprints202009.0232.v1

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Notes

The authors declare no competing financial interest.

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