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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 with multiple technical and economic advantages. Coupled with the simplicity and sustainability of the extraction processes suggested, the high chemical and physical stability of this polymethoxylated flavonol 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 for 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 a 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 and 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 were 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, some of us identified and isolated limocitrol 3-*O*-6″-[3-hydroxyl-3-methylglutaryl)])-*β*-D-glucopyranoside, namely a flavonol glycoside called yellow #15, in the ethanol extract of *Citrus limon* fruit zest [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].

2. Technical and Economic Aspects

Lemon yellow #15 was isolated from the ethanol extract of dried *Citrus limon* zest obtaining 4 mg of pure dye starting from 132.9 g dried zest obtained after lyophilization of 305.6 g raw peel [8]. The lyophilized zest was extracted with EtOH in a sonicator for one hour, after which the extract underwent column chromatography according to the procedure recently described [8].

Eventually, yellow #15 was isolated via chromatography on Sephadex LH-20 column and HPLC. The dye easily dissolves in water from which it can be isolated via simple freeze-drying as a yellow powder (Figure 1).

(**a**) (**b**)

Figure 1. Lemon-derived yellow #15 in aqueous solution (**a**), and in powder form (**b**) (pictures by Dr. X. Chen and Prof. M.T. Hamann, The Medical University of South Carolina).

Following careful molecular structure and configuration assignment based on NMR spectroscopy, optical rotation measurements, and comparison of the experimental CD with calculated electronic circular dichroism (ECD) spectral data, the colorant was shown to be the limocitrol glycoside esterified with 3-hydroxymethylglutaric acid (Figure 2), limocitrol 3-*O*-6″-[3-hydroxyl-3-methylglutaryl)])-*β*-D-glucopyranoside.

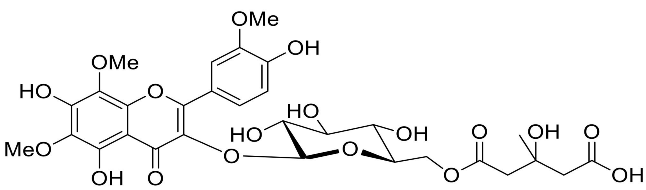


Figure 2. Chemical structure of yellow #15: limocitrol 3-*O*-6″-(3-hydroxy-3-methylglutaryl)-*β*-D-glucopyranoside.

This explains why yellow #15 is considerably more soluble in water than curcumin, and more soluble than 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].

The proposed scale-up procedure to extract yellow #15 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 3) [8]. Following addition of the extracts to absolute (200 Proof) EtOH, a lemon EtOH fraction precipitated out of solution (Figure 3b) concentrated in highly stable and water-soluble food dye yellow #15. The colorant can be used as such to impart the yellow color to foods and aqueous beverages.

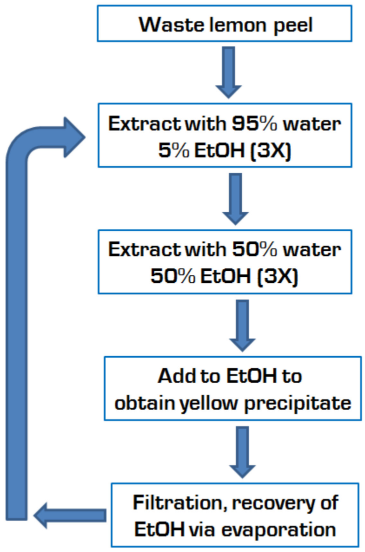
(**a**) (**b**)

Figure 3. Ground lemon zest extracted three times (1,2,3) with 95:5 H_2O:EtOH followed by three new extraction rounds (4,5,6) with 50:50 H_2O:EtOH (**a**). The solid precipitate upon addition of the extracts to pure EtOH (**b**). (Pictures by B. Forest and Professor M.T. Hamann, The Medical University of South Carolina).

Displayed in Scheme 1, the hydroalcoholic extraction involves a first extraction of 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. Valued ethanol can be efficiently recovered by evaporation under reduced pressure and reused in subsequent extraction runs.

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 solvent [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].



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

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 HC-based process enables extracting 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 flavonoids imparting to lemon peel its yellow color are dissolved in water (Figure 4) from which they can be easily isolated using an optimized version of the chromatographic process demonstrated on laboratory scale with the dye extracted with aqueous ethanol [8].

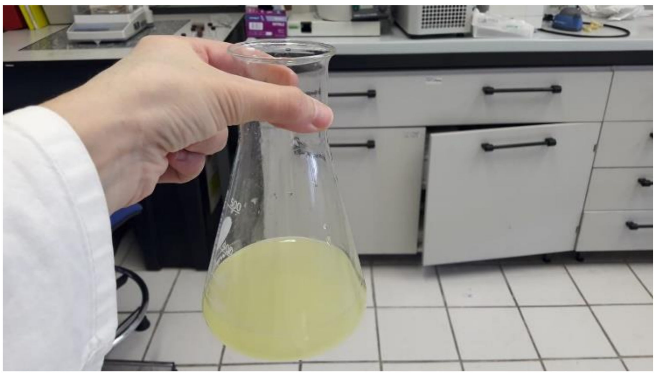


Figure 4. The yellow aqueous solution following hydrodynamic cavitation of waste lemon peel (image courtesy of Dr. M. Pagliaro, CNR).

It is also important, in light of forthcoming practical applications, 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 lemon yellow #15 from waste lemon peel either using the hydroalcoholic extraction route or via controlled hydrocavitation in water would be (i) labor, and (ii) electricity needed either to recover the bio-based solvent (EtOH) or to power the

HC reactor. The latter cost today can be significantly reduced with a relatively low investment cost 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 the world's countries [18].

Data in Table 1 show that growth in lemon production between 1980 and 2016 (+218%) outpaced that of citrus from 61 to 146.5 million tonnes (+140%) [19]. Out of 17.5 million tonnes harvested in 2016, 3.1 million were exported (19%). Followed by Spain (688,256 t in the 2016/2017 season) and Turkey (451,911 t), Mexico (733,918 t) is the world's leading country in terms of lemon production.

Table 1. Amounts of lemons harvested globally in 1980 and 2016 (adapted from Ref. [19], with kind permission).

Year	Amount (Million Tonnes)	Share of Citrus Production
1980	5.2	8%
2016	17.5	13%

The cost of the raw material (waste lemon peel) available as a by-product of lemon juice (and lemon oil) manufacturing plants is extremely low (and often zero, with citrus companies saving on waste disposal costs) whereas demand and production of lemons in the last two decades, driven by a powerful megatrend demand for healthy "naturals" in food, beverage, cosmetic, personal care, and nutraceutical products, have been growing steadily. Alone, the main lemon producing countries are expected to report an increase in production by over 1.2 million tonnes in the 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 at about 50% of 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 #15 natural colorant. The dye, a water-soluble limocitrol glycoside, is a flavonoid suitable as an industrial alternative to both synthetic and 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.

A comparison of various extraction procedures in terms of yield, purity, and cost will be carried out including also other extraction routes such as supercritical carbon dioxide that, in the case of citrus flavonoids, in the presence of 85% ethanol as modifier, affords significantly higher yield in the amount of flavonoids extracted, and significantly shorter extraction times [22].

Coupled with the ease and greenness of the extraction processes suggested, the low cost and abundance of waste lemon peel, alongside the enhanced stability of the natural colorant and the health benefits of citrus flavonoids (cardioprotective, chemopreventive, and neuroprotective effects) [23], support industrialization of this new bioeconomy production.

In the circular economy approach applied to citrus, resulting in what Siles López, Li, and Thompson in 2010 called "the citrus biorefinery" [24], production of the yellow biocolorant would take 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 of affording all the aforementioned bioproducts, if possible in one pot and with high energy efficiency.

Once said bio-based production will be industrialized, most likely directly at large citrus processing plants, this polymethoxylated flavonoid derived from lemon 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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