ABSTRACT

Pectin, a non-starch polysaccharide, is extracted as a byproduct from agro-processing and has various applications in the pharmaceutical and food industries, including drug delivery, gelling, stabilization, and thickening in food systems. This study examines pectin extraction from Assam lemon, the formulation of the pectin complex, and its application in food, considering physical, chemical, functional, and rheological aspects. Initially, the optimal parameters for conventional (CE), microwave-assisted (MAE), and ultrasound-assisted (UAE) extraction were evaluated to maximize pectin output from Assam lemon (*Citrus limon*) peels. The extraction temperature/exposed power/amplitude, time and solid-liquid ratio were optimized for CE, MAE and UAE, respectively using the Box-Behnken design. The independent variables for standardizing pectin extraction conditions significantly influenced yield. The highest pectin yield of 32.17% was attained using UAE, whilst CE and MAE produced yields of 16.56% and 19.61%, respectively. The pectin acquired under optimal conditions was characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and chemical analysis. The findings indicate that MAE and UAE possess the capability for the effective extraction of pectin-rich extracts for diverse applications.

A further objective was to focus on iron deficiency, a global health concern, by creating a functional element from pectin derived from Assam lemon and enhancing iron content through the pectin iron complex (PIC). Pectin was incubated with iron chloride hexahydrate (0.90–1.80 mM) for 180 hours to optimize the complexation conditions, determining its optimum value as 1.36 mM. The PIC was analyzed utilizing FTIR, XRD, SEM, rheological, thermal and chemical assessments. The bioavailability of iron and its absorption in the PIC were evaluated by *in vitro* simulated digestion and Caco-2 cell monolayers. The bioaccessible iron content in the produced PIC throughout the intestine phase was 5.34±0.16%, which was minimal in pectin. The bioaccessible iron absorption in the PIC was determined to be 2.93±0.03%.

Moreover, the developed PIC was used in the formulation of fruit leather and tested its shelf-life stability. The application of a mixture design approach to develop an optimal PL formulation by using pectin complex (PIC) along with pineapple pulp and sugar was used. From mixture design, sixteen experimental runs were conducted to evaluate the effects of

independent variables on the antioxidant activity, total phenolic content, browning index and sensorial acceptance. The optimal PIC based pineapple leather (PL) was prepared with the composition of pineapple pulp (90.588% w/w), PIC (1.833% w/w) and sugar (7.579% w/w). To further investigate its shelf life, the optimum PL was packed and kept for 35 days at three distinct temperatures (4°C, 15°C, and 25°C). Every seven days, moisture content, pH, water activity, browning index, total phenolic content, antioxidant activity, texture, browning index and microbial studies in PL were examined. Based on the storage studies, PL formulated using PIC exhibited adequate stability at all temperatures during 35 days and could be considered safe for consumption.

Keywords: Assam lemon; pectin; Microwave assisted extraction; Ultrasound-assisted extraction; Pectin-iron complex; *In-vitro*; Caco-2 cells; Bioaccessibility; Fruit leather