



# Evaluation of ultrasound based sterilization approaches in terms of shelf life and quality parameters of fruit and vegetable juices



Paramjeet Khandpur, Parag R. Gogate \*

Chemical Engineering Department, Institute of Chemical Technology, Matunga, Mumbai 400019, India

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## ABSTRACT

The present work evaluates the performance of ultrasound based sterilization approaches for processing of different fruit and vegetable juices in terms of microbial growth and changes in the quality parameters during the storage. Comparison with the conventional thermal processing has also been presented. A novel approach based on combination of ultrasound with ultraviolet irradiation and crude extract of essential oil from orange peels has been used for the first time. Identification of the microbial growth (total bacteria and yeast content) in the juices during the subsequent storage and assessing the safety for human consumption along with the changes in the quality parameters (Brix, titratable acidity, pH, ORP, salt, conductivity, TSS and TDS) has been investigated in details. The optimized ultrasound parameters for juice sterilization were established as ultrasound power of 100 W and treatment time of 15 min for the constant frequency operation (20 kHz). It has been established that more than 5 log reduction was achieved using the novel combined approaches based on ultrasound. The treated juices using different approaches based on ultrasound also showed lower microbial growth and improved quality characteristics as compared to the thermally processed juice. Scale up studies were also performed using spinach juice as the test sample with processing at 5 L volume for the first time. The ultrasound treated juice satisfied the microbiological and physiochemical safety limits in refrigerated storage conditions for 20 days for the large scale processing. Overall the present work conclusively established the usefulness of combined treatment approaches based on ultrasound for maintaining the microbiological safety of beverages with enhanced shelf life and excellent quality parameters as compared to the untreated and thermally processed juices.

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## 1. Introduction

Fruits and vegetables contain many essential compounds such as nutrients, vitamins and minerals which are beneficial for health and overall well-being of human body. The consumption of fresh fruits and vegetables has limitations and food spoilage due to the microbial growth is a major cause of economic loss in the food industry. There have been several reports on the cases of food borne illness including food poisoning caused by the uptake of the spoiled fruit/vegetables and other products in India and other third world countries [1–4]. Several extrinsic factors related to the environment and intrinsic factors such as the conditions in which food is stored influence the growth of micro-organisms in the stored food products. Production of processed products with higher shelf life without significant use of harmful preservatives is very important and can result in better economic considerations. Continuously increasing consumer demand for packaged foods and

beverages with fresh like attributes has encouraged food industries to adopt innovative food processing technologies. Today thermal treatment is the most extensively practiced pasteurization and sterilization approach for the inactivation of harmful enzymes as well as microorganisms (that can lead to spoilage or unnecessary changes in quality such as browning) in the food industry to produce safe food with increased shelf life [5]. Although strict practices and thermal processing for controlling the safety of fruit and vegetable juices have been implemented, the existing operations at commercial scale are not completely efficient especially considering the quality of the processed juices. There are significant quality changes in the thermally processed juices, which results in lower consumer preference and significant interest has been generated to direct research into developing non-thermal processing approaches which can maintain the produce safety as well as the quality of the processed product. Using non-thermal technologies can give an advantage in terms of good quality parameters for the processed beverage from fruits and vegetables and also can enable to transmit the nutritional and functional benefits of fruits and vegetables to a wider population. Thus,

\* Corresponding author.

E-mail address: [pr.gogate@ictmumbai.edu.in](mailto:pr.gogate@ictmumbai.edu.in) (P.R. Gogate).

development of commercially feasible method(s) to minimize the microbial contamination, nutritional component loss, and occurrence of offensive flavor and odor components in the beverage is very important. Use of ultrasound (US) or ultraviolet (UV) based treatment is one of the promising approaches that can be applied for the inactivation of microorganisms as per the US Food and Drug Administration's requirement of a 5 log reduction with adequate maintenance of the quality. The current work deals with understanding the efficacy of ultrasound based treatment approaches (only ultrasound, combination of US and UV as well as combination of US and orange peel crude extract) for treating selected fruit and vegetable juices in terms of the microbiological quality assessment and shelf life studies both in terms of microbial growth and retention of quality of the processed beverages.

Power ultrasound offers as a potential technology to destroy the microorganisms generally found in fruit and vegetable juices. Controlled application of ultrasound can be a promising food preservation approach that inactivates microorganisms without having harmful effects on the nutrition, quality, sensory and aesthetic attributes of food [6–8]. The microorganism inactivation action of ultrasound is usually attributed to the cavitation effects such as conditions of intense localized pressure and temperature pulse as well as high intensity shear and turbulence. These cavitation effects can lead to the breakage of cell walls as well as damage the DNA finally leading to deactivation of different microorganisms [7,9–11]. Ultrasound has been commonly applied for the inactivation of different pathogenic and spoilage micro-organisms in variety of juices such as apple cider [12], orange juice [13–15], guava juice [16] and tomato juice [17].

Ultraviolet light based irradiation can also be effectively applied for microbial disinfection of different fruit and vegetable juices. UV-C light has good microcidal properties and therefore it has beneficial effects in food processing for preventing microbial contamination. The UV irradiation affects the DNA of exposed microbial cells based on the formation of thymine dimer in the DNA of the microorganisms and hence the ability of microorganisms to grow is destroyed [18].

The extracts obtained from the citrus fruit residues such as orange peels also have antimicrobial properties and hence have been investigated in the present work as a supplementary approach to ultrasound. The waste products obtained during the processing of citrus fruits for juice production [19] can be a sustainable source for recovery of many important natural active ingredients. For example, significant quantum of flavanones and many polymethoxylated flavones [20] are present in the peel of Citrus fruits and these flavonoids offer significant prospective applications mainly due to the range of biological activities [21,22]. Citrus peel extracts are usually composed of citrus peel oils, which have inhibiting action on the Gram positive and Gram negative bacteria, different types of yeasts, molds and food poisoning bacteria [23]. A novel approach of using the recovered active ingredients from citrus peel wastes in combination with ultrasound for juice preservation has been attempted in the present study.

In summary, the overall goal of the current study is to conduct a systematic investigation for establishing the benefits obtained by using ultrasound based treatment in terms of the enhanced shelf life with analysis of the microbiological quality parameters and the contents of the nutritional compounds. The importance of the work is reflected in the fact that the effect of the combined approach of ultrasound and ultraviolet as well as ultrasound and orange peel extract on the microbial parameters of the processed juices have been investigated for the first time to the best of our knowledge.

## 2. Materials and methods

### 2.1. Juice treatment

Juices obtained from fruits such as oranges, sweet lime and vegetables such as carrots and spinach were used for this study. The juices were extracted as per the detailed process described earlier [24] and then subjected to different sterilization treatments. Conventional thermal treatment was carried out by heating the juices at 80 °C temperature for 10 min using a water bath. Ultrasound treatment was carried out using ultrasonic horn system (diameter of probe as 10 mm). The ultrasonic operation was set at the screened optimal processing variables of 20 kHz, 100 W (power density of 0.4 W/ml), 50% duty cycle, treatment time as 15 min and the temperature of less than 30 °C. For the combined operation of ultrasound along with ultraviolet irradiations, an additional effect was created by exposing the juice sample to ultraviolet light simultaneously along with ultrasound. The juice was placed in a quartz beaker and the ultrasound probe was immersed in the beaker. Magnetic stirring was also used to achieve uniform mixing of the contents. On either side of the beaker, 2 UV lamps with 8 W power dissipation emitting light in the 200–300 nm wavelength range with a maximum at 254 nm were placed vertically parallel to each other. The entire set up was covered using a box and black cloth so that the radiations do not penetrate out. The germicidal action of ultraviolet light is known over the wavelengths of 200–280 nm and usually the dominant wavelength is 254 nm. As the presence of solid particles are expected to provide a protective shield for the microorganisms as well as reduce the available intensity of the incident light for treatment [25], the juices were filtered before subjecting to ultrasound–ultraviolet treatment in order to ensure that the juice is free from any suspended particles. Scale up studies for the specific case of spinach juice was conducted using Ultrasonix reactor having a capacity of 7 L, power dissipation of 150 W and frequency of 36 kHz.

### 2.2. Combined treatment of ultrasound and crude extract of orange peel essential oils

A novel approach of using the extracted antimicrobial metabolites from the orange peels in combination with ultrasound was explored for the first time. The orange peels were dried and antimicrobial metabolites were extracted using the solvent extraction procedure. Extracts were subjected to antibacterial and antifungal susceptibility assay using the agar disc diffusion method. Later crude essential oils extracted from orange peels were combined with ultrasound and this hurdle treatment concept has been evaluated as a sterilization approach focusing on the effects on the microbial and nutrient parameters during the storage.

#### 2.2.1. Preparation of peel extracts from orange (*Citrus sinensis*)

Orange peels left out after juice extraction were collected, cut into small pieces and sun dried over a period of 6–7 days. The samples obtained after sun drying were grounded using a series of crushing steps to yield a finely powdered form, which was stored separately in air tight bottles. Different solvents such as water, methanol, ethanol, ethyl acetate, acetone, chloroform and hexane were used for extraction of the active ingredients from the obtained orange peel powder. In any run, 5 g of the orange peel powder was soaked in 100 ml of solvent under ambient conditions for 48 h in an orbital shaker unit. The obtained mixture was filtered through a Whatman No. 1 filter paper for the removal of particulate matter and the obtained extracts were evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator. Yield of the extract was weighed on the weighing balance. The extracts

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