# ABSTRACT

Pineapple (*Ananas comosus* L.) puree, usually produced by crushing fresh pineapple, is used as a starting material for juices, beverage and other fruit servings. It is a rich source of micronutrients, phytochemicals and beneficial protease, bromelain (BRM) which has numerous health benefits. Several enzymes like polyphenoloxidase (PPO), peroxidase (POD) and pectinmethylesterase (PME) together with some spoilage microbes are responsible for deteriorating the quality of the pineapple puree.

The effect of pH (3.0, 3.5 and 4.0), total soluble solids (TSS, 10-14 °Brix) and high pressure process treatments viz. pressure (*P*, MPa), temperature (*T*, °C) and dwell time (*t*, min) on the inactivation of PPO, POD, BRM and PME in pineapple puree was studied within the range of 100-600 MPa/20-70 °C/0-30 min. The estimated kinetics parameters suggested that the puree sample of pH 3.5 and TSS of 10-14 °Brix was effective for HPP within the experimental domain for the maximum (77%) retention of BRM. After selecting the suitable pH and TSS, the effect of process variables (*P*, *T* and *t*) on the enzyme activity, microbial population, color, biochemical and nutritional properties of the puree was studied within the domain of 200-600 MPa; 30-70 °C; and 0-20 min. At 600 MPa/20 min, the maximum loss in ascorbic acid (AA) was 20 and 25% at 60 and 70 °C, respectively. Total phenolic content (TPC), total antioxidant capacity (TAC) and total flavonoid content (TFC) were stable (loss of < 10%) up to 50 °C at all the pressure levels (200-600 MPa/0-20 min) followed by a decrease at temperatures > 50 °C.

The most baro-resistant microbiota and enzyme were yeast and mold (YM) group and PPO, respectively. Isobaric enzyme inactivation followed an order ($n$) < 1 within 200-600 MPa/30-70 °C. Weibull model was able to describe the non-linearity of the microbial survival curves during HPP. The maximum *PE* values for the microbiota were obtained at 600 MPa/50 ºC. As confirmed by the shape parameter (β) of the survival curve, the tailing effect was obtained in case of YM (β = 0.85); whereas a shouldering effect (β > 1) was observed for the remaining microbial groups viz. psychrotrophs (PC), aerobic mesophiles (AM), total coliform (TC) and lactic acid bacteria (LAB). The maximum inactivation of PPO, POD and BRM were 92, 98 and 77%, respectively obtained at 600 MPa/70 ºC/20 min. Two different empirical models were developed describing inactivation rate ($k$) of microflora or enzyme during HPP as a function of *P*, *T*, activation energy ($E_a$, kJ·mol$^{-1}$) and activation volume ($V_a$, mL·mol$^{-1}$) values. The process conditions were optimized further within the domain of 400-600 MPa, 40-60 ºC and 10-20 min using RCCD and RSM.

The optimum HPP condition was 600 MPa/50 ºC/13 min having the desirability value of 0.604. The optimally processed sample (S2) had 44% PPO activity, 47% BRM activity, 93% TAC and 85% AA with a $\Delta E^*$ value less than 2.5. A ten-fold reduction in PPO activity was obtained at 600 MPa/70 ºC/20 min (sample S3) and 0.1 MPa/95 ºC/12 min (sample S4); however a higher magnitude of temperature (like 70 and 95 ºC) had detrimental effects on the bioactive components and color parameters. The similarity values (sensory acceptance) obtained through Fuzzy mathematical approach for S1, S2, S3 and S4 were 0.96, 0.93, 0.83 and 0.78, respectively. The storage stability of samples S1, S2, S3 and S4 packed in multi-layered (ML) polyethylene terephthalate and ethylene vinyl alcohol (EVOH) films was evaluated upto 120 days at 5, 15 and 25 ºC. The optimized puree (S2) packed in EVOH film had the shelf-life of 110, 50 and 20 days at 5, 15 and 25 °C, respectively. In S3 and S4 with EVOH film, the count for AM and YM were < 6 Log·cfu·g$^{-1}$ upto 120 days at 5 °C; whereas, S1 spoiled after 2, 5 and 7 days at 5, 15 and 25 °C, respectively.



**Keywords:** Pineapple puree, high pressure processing, enzyme inactivation, microbial destruction, kinetics, optimization, Fuzzy logic, shelf-life.