High pressure and Temperature Effects on Enzyme Performance in Orange Juice and Tomato Puree

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Abstract- *High pressure (200-400MPa) (or high hydrostatic pressure(HHP) or Ultra high pressure(UHP)) treatments combined with heat treatment (20-60^oC) effects on enzymes like peroxidise (POD), polyphenol oxidase (PPO), and pectin methyl esterase (PME) activities of fruit derived products like juices and purees. Assays were carried out on fresh orange juice and tomato puree. Pressurisation / depressurisation treatments caused a significant loss of orange POD (50% up to 400Mpa at 35^oC and POD activity (25%) up to 400Mpa for 15min at 35^oC and PME (25%) up to 300MPa, while some activation is observed between 200-350MPa for POD at room temperature . Optimum inactivation of POD was using 400Mpa at 35^oC for 15min. Combination of high pressure and temperature effectively reduced PME activity (35%). Combination of 200 MPa/25^oC showed 10% inactivation in PPO activity in tomato puree.*

Keywords- High pressure or Ultra high pressures(UHP); peroxidise(POD); polyphenol oxidase(PPO); pectin methyl esterase(PME)

I. INTRODUCTION

Fruits and vegetables are considered as protective foods and are integral part of healthy food. Fruits preserved in the form of purees and juices are very popular as these products are convenient to consume and form a part of many formulations, beverages, etc., [6].The main problem in fruit processing industry is these products are susceptible for spoilage [3]. Besides microbial spoilage, quality degradation due to endogenous enzyme activity is also a problem to be taken care of. In whole fruits, the enzymes and the substrates are confined in separate compartments. But in squeezed purees and juices, this separation is obliterated which offers enzymes to come in contact with substrates resulting in the products with poor quality attributes[7]. Oxidative enzymes like polyphenoloxidase (PPO), peroxidase (POD) etc., are responsible for the deterioration of color, flavor, and nutritional value [9]. Pectinolytic enzymes like pectinmethylesterase (PME) etc., are involved in the breakdown of pectin network surrounding the cellulose backbone of the cellwall and thus produce low viscous products with modified color and other organoleptic properties [5]. The activity of PME thus results in pectin modification and subsequent changes in texture of the puree or juice.

High pressure treatment for food preservation is considered as a modern method. Earlier, thermal treatments for food preservation dominated food processing industry. However, this traditional heat treatment method causes undesirable changes in texture, flavour, odour and even it can destroy heat sensitive nutrients such as vitamins. As demand for microbiologically safe, minimally processed, additive free and nutraceutically important food products are day by day increasing, an alternative non-thermal food processing technology with good quality attributes are actively being investigated[10][2].

A number of non-thermal technologies are in use like, ionisation, electron beam, UV, pulsed X-ray, ultrasound, high pressure carbon dioxide(HPCD), high hydrostatic pressure(HHP) [11]. Of all these processing methods high pressure treatment or high hydrostatic pressure treatments are well investigated on its efficiency in terms of enzyme activity performance. This treatment do not effect foods adversely like that in thermal treatment thus allows retention of physical, nutritional and sensory qualities closer to its natural state.

Studies on effect of pressures on proteins and enzymes thus showed interest among research community and interest in application of high pressure treatment for food preservation is increased. Effects of high pressure above 300MPa treatment on enzymes may be attributed to irreversible denaturation at room temperature on the protein structure ; while reversible changes occur at lower pressures below this [8][13]. But loss of enzymatic activity due to application of high pressures depends on type of juice, type of enzyme, nature of substrates, temperature and time of processing. This is due to the fact that some enzymes are more pressure stable. For example, certain enzymes can with stand high pressures upto 1000 Mpa [13]. For full inactivation of certain enzymes pressure in combination with heat are needed.

Our objective in this study was to determine effects of high pressure treatments up to 400 MPa combined with mild heat treatments up to 60^{o}C on peroxidase (POD; EC 1.11.1.7), polyphenol oxidase (PPO; EC 1.10.3.1), and pectin

methyl esterase (PME; EC 3.1.1.11) activities in orange juice and tomato puree.

II. MATERIALS AND METHODS

2.1 High pressure treatments

The high pressure treatments was carried out according to the described method with slight modifications (CANO et al., 1997).

Oranges (*Citrus aurantium*) and tomatoes (*Lycopersicum esculentum*) were obtained from local commercial market. Fruits for processing were selected which are disease free. Tomato puree is obtained by homogenization using a blender. Squeezed orange juice was freshly prepared by juice extractor. Samples were placed in polyethylene bottles (250ml) and then introduced into the pressure unit, containing a maximum pressure of 500 MPa and with a maximum temperature of 95^{o}C. Pressure was increased and released at 2.5Mpa/s. The pressure treatment time was 15 min constant for orange juice and 5 min constant for tomato puree and temperature of immersion medium (initial sample at atmospheric pressure; 20^{o}C) was varied between 20^{o}C to 60^{o}C. After pressure treatment samples were immediately analysed or stored at -40^{o}c for enzyme activity determination.

2.2 Quality determinations

Soluble solids of tomato and orange fruits were determined using a digital refractometer. Results were reported as ^{o}Brix at 20^{o} C. For titrable acidity, the puree or juice of fruits were macerated and 10g samples were accurately weighed into beakers. Distilled water (40 mL) was added to each sample. The resulting mixture was titrated with 0.1N NaOH to pH 8.0 monitored with a pH meter. The results were expressed as g citric acid/100g sample. The pH of samples was determined before titration.

2.3 Biochemical analysis

The enzyme extracts for determination of peroxidase and polyphenol oxidase were made by homogenization of 10g of each sample with 20–25 mL of 0.2M sodium phosphate buffer (pH 6.5) (containing 4% (w/v) insoluble polyvinylpolypirrolidone (PVPP) and 1% (v/v) Triton X-100 (for tomato samples) in a homogenizer with external cooling, for 3 min with stop intervals each 30 sec. The extract for determination of pectin methylesterase activity in orange juice was made by homogenization of 10g sample with 30 mL of 2M sodium chloride (pH 7.5).

Peroxidase activity was assayed spectrophotometrically using aliquots (0.05 mL) of extract and a reaction mixture containing 2.7 mL 0.05M sodium phosphate buffer (pH 6.5) with 0.2 mL 1% (w/v) p-phenylenediamine and 0.1 mL 1.5% (w/v) hydrogen peroxide. The oxidation of p-phenylenediamine was measured using a spectrophotometer at 485 nm ,25^{o}C.

Polyphenoloxidase activity was assayed using aliquots (0.1 mL) of extract and 3.0 mL of a solution of 0.07M catechol in 0.05M sodiumphosphate buffer (pH 6.5). The reaction was measured with the spectrophotometer at 420 nm at 25^{o}C.

Pectin methylesterase activity was assayed using aliquots (0.1 mL) of extract and a reaction mixture composed of 2.0 mL 0.5% (w/v) pectin (pH 7.5), 0.15 mL 0.01% (w/v) bromothymol blue in 0.003M potassium phosphate buffer (pH 7.5) and 0.75 mL distilled water. The reaction was measured spectrophotometrically at 620 nm at 25^{o}C.

All enzyme activities were determined by measuring the slope of reaction. The enzyme activity unit was defined as the change in absorbance/min/g fresh wt of sample.

III. RESULTS AND DISCUSSION

Initial enzyme activities in freshly prepared fruit-derived products (Table 1) showed similar peroxidase activity (3.19 ΔOD/min/g f.w.) in both orange juice and tomato puree. Polyphenol oxidase activity in tomato puree was found twofold more (0.42 ΔOD/min/g f.w.) comparing to orange juice (0.23 ΔOD/min/g f.w.). Whereas, Pectin methylesterase activity in control orange juice was noticed high 6.3 ΔOD/min/g f.w comparing to tomato puree (Table 1). These experiments are carried out according to the methods described above. Peroxidase activity in orange juice was increasingly inactivated up to 400 MPa (50% inactivated), for treatments carried out at temperature (35^{o}C) and 15 min of pressurization. UHP treatments above 300 MPa at room temperature slightly increased POD activity.

PME activity of fresh squeezed orange juice was a 6.3 ΔO.D./min/g f.w. This initial activity was reduced to 25% by UHP treatment at 200 MPa/30^{o}C. However, at room temperature UHP treatments produced an activation of PME activity in the 200–400 MPa range. Increased processing temperature strongly affected this activation (Fig. 2). Only combinations of low pressures and mild temperatures inactivated PME in orange juice. According to our results the POD inactivation was observed to be equal to PME inactivation after 350 MPa and above 30^{o} C temperature.

Scientists previously reported that the effects of UHP treatments on POD and PPO activities in cell-free extracts showed that peroxidase was quite resistant to pressurization below 900 MPa (1 min treatment), the complete loss of enzyme activity was achieved only at 900 MPa, while activation was observed for treatments in the 300–500 MPa range [1].

PPO activity in tomato puree showed an inactivation at 400 MPa at 25°C for 5min. The enzymes of other commodities such as potatoes and apples also were activated by pressure treatment and, the tissues darkened rapidly after pressurization. They suggested that the PPO activation in pressurized plant products would follow the kind and number of isoenzymes of their PPO enzymatic system. Isoenzymes could show different optimum pH values, and it is not clear whether the activity would be increased by pressurization. In our results, the activation of PPO activity did not produce darkening in fruit puree, but further stability studies will be carried out to establish the possibility of PPO regeneration during chilled storage.

The influence of soluble solids content of samples on inactivation of enzymes has been reported [12]. Increased soluble solids protect PME against pressure as well as heat inactivations. Our results, tomato puree had the higher soluble solids content. This was considered a significant factor to contribute to the initial resistance against inactivation of its POD and the PME activation at medium pressures. Also, reported results showed that high pressure treatment could partially and irreversibly inactivate PME, which did not reactivate during storage and transportation [12]. This could be important for achieving commercial stability of fruit-derived products with a nearly fresh quality.

3.1 Industrial Applications of HHP

HP technology has become a commercially implemented technology in fruit juice processing, spreading from its origins in Japan to the USA and Europe, and now Australia, with worldwide utilization increasing almost exponentially since 2000. In the U.S., Genesis Juice Corp.® processes different types of organic juices by HHP. Regarding processing conditions, treatments are optimized at a pressure level of 600 MPa in combination with moderate heat. In addition, due to the special characteristics of fruit juices, (nutritional components, flavor) and the perception by the consumer as a healthy food, quantities ranges can be produced to satisfy current consumer demand considering the current capacities of industrial equipment.

3.2 Conclusion & Future Perspectives

The potential of high-pressure technology has been proven to be good in the context of inactivation of enzymes in fruit juices & purees, predicting the extent of inactivation for an enzyme. In the last decade, some of the studies have reported that complete inactivation of a resistant enzyme in a specific fruit juices & purees was not possible even up to 600 MPa. However, this problem can be solved by applying some more amount of mild heat treatment with pressure. High-pressure–treated products will attract consumer attention for their “natural” or “fresh-like” characteristics. But it should also be noted that initial installation of any high-pressure equipment may add extra cost to the product.

Future studies should focus on the mechanism behind the high-pressure–induced inactivation of fruit enzymes. The conformational modification in the native structure of an enzyme during pressurization should be found out. Reactivation, inactivation, and inhibition mechanisms of the enzyme induced by pressure and/or temperature may also be seen as a priority area. Cost-effective and productive high-pressure processes with respect to enzyme inactivation in fruit juices and purees have to be optimized keeping in mind consumer awareness and their willingness to pay for high-pressure–processed fruit products.

Table 1- Physicochemical and biochemical characterisation of samples before pressurization

Characteristic	Orange juice	Tomato puree
Titrable acidity (g citric acid/100 g f.w.)	0.90 ± 0.04	0.47 ± 0.01
pH	3.70 ± 0.05	4.2 ± 0.02
Soluble solids (°Brix at 20°C)	11.34 ± 0.10	20.4 ± 0.10
Total solids (mg/100 g f.w.)	12.70 ± 0.08	11.2 ± 0.90
POD activity	3.20 ± 0.10	3.20 ± 0.01
PPO activity	0.23 ± 0.02	0.42 ± 0.02
PME activity	6.3 ± 1.30	5.0 ± 1.10

Table 2- Levels of variables in fruit-derived products UHP processing

Pressure (MPa)	Temperature(°C)
50	20.0
100.0	25.0
225.0	40.0
350.0	55.0
400.0	60.0

Table 3- High pressure inactivation of enzymes in orange juice and tomato puree

Food product	Enzyme	HHP condition as MPa/min/°C	Inactivation achieved	other observation
Orange juice	POD	250-400/15/35	50% (400/15/35)	Activation Up to 200-350MPa at room temperature
	PPO	100-400/15/25	25% (400/15/35)	-
	PME	300-400/15/30	25%-40% (300/15/30)	
Tomato puree	POD	100-400/5/25	63%	-
	PPO	100-400/5/25	70% (400/5/25)	-
	PME	300/2/20	25%-35% (300/5/30)	Activation up to 200 MPa

ACKNOWLEDGEMENT

This work was financially supported by UGC-SERO with wide No.F MRP-6074/15(SERO/UGC)

The authors would like to thank Dr.CH.V.Purushottam Reddy, Correspondent , Chaitanya Colleges, Kishanpura for providing lab facility for research work and Dr. K. Veera Venkataih, Principal, Chaitanya P.G.College for his encouragement to take up this work.

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