

# Combined effect ozonation and ultrasonication on rheological and thermal properties of rice starch in aqueous phase

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## RESEARCH ARTICLE

### Abstract

A combination of ozonation and ultrasonication was applied to rice starch in aqueous phase. A Bohlin rheometer was used to obtain flow behaviour and consistency indices. A differential scanning calorimeter was used to measure the gelatinisation properties (onset, peak and conclusion gelatinisation temperatures and enthalpy of gelatinisation). It was observed that all starch samples exhibited non-Newtonian behaviour (i.e.  $n < 1$ ). The highest viscosity values (i.e. 0.5 Pa.s) and the highest shear-thinning effect (i.e.  $n = 0.4$ ) were observed for ultrasonicated starch sample. The consistency index for native rice starch increased after ultrasonication. There were no significant differences ( $P < 0.05$ ) between the consistency index and apparent viscosity of samples treated with ozonation and ultrasonication with no regards of treating sequence. This showed that the order of treatment (i.e. ozonation and ultrasonication) does not have any significant effect on the flow properties of rice starch studied under the conditions applied in this research.

**Keywords:** non-thermal process, oxidation

### 1. Introduction

Unmodified native starches usually do not present functional properties necessary for the industrial applications. Therefore, modified starches are generally used to control flow behaviour, physical and sensory properties of foods (Goze *et al.*, 2016). Modification of starch can be performed by physical, chemical and enzymatic methods.

Oxidised starches are used for lower viscosity, improved stability and binding properties, reduced retrogradation and gelling tendencies, all of which are leading to better cooking properties and storage abilities (Castanha *et al.*, 2017; Vanier *et al.*, 2017). Acidic bromate, hydrogen peroxide, sodium hypochlorite and potassium permanganate are common agents used in the oxidation of starch. Ozone is regarded as a powerful oxidant which can be used safely in both gaseous and aqueous phase. Ozone is also defined as GRAS (generally recognised as safe) by the US Food and Drug Administration (FDA, 1997, 2001). Ozone is considered as an alternative oxidant to the chemicals mentioned above since it is able to oxidise starch at low temperatures without any problem of effluents. Ozone oxidation is sometimes

regarded as a green technology since ozonation does not leave any hazardous residues in food products and it is quickly converted to atmospheric oxygen (An and King, 2009).

Ultrasound treatment of food components is reported to lead improved quality attributes with shorter processing times. The microbial populations of food products can also be reduced by ultrasonication (Zhu, 2015). Ultrasound frequencies at which human beings cannot hear (i.e.  $> 16$  kHz) are generally applied in the food applications. Ultrasonication may change the structure of starch by creating high temperatures, local shear forces and free radicals (Carmona-Garcia *et al.*, 2016). Ultrasound technology is sometimes regarded as environmentally friendly green technology in the food industry (Chang *et al.*, 2017; Zhu, 2015). The main idea behind this work was to see the combined effects of ozonation and ultrasonication on starch properties. Since the effects of ozonation and ultrasound processing have different effects on their own, we would like to examine what would happen when the two process are applied in series. Also, it would be useful to know whether the order of treatment has an effect on rice starch

properties (i.e. ozonation followed by ultrasonication and ultrasonication followed by ozonation). The combination of two different processes would lead to starch samples with rheological and thermal properties which could be different from those obtained when processed by ozonation and ultrasonication only. In the case of starch, most research has been focused on the effects of ozonation and ultrasonication when applied separately (Vanier *et al.*, 2017; Zhu, 2015). Our previous work on ozonation of corn, wheat and potato starch showed that ozone application caused radical changes in the rheological, thermal and structural properties of samples (Çatal and İbanoğlu, 2012, 2014). Limited data is available on the combined effect of ozone and ultrasound treatment on starch. In one of such research, Chong *et al.* (2013) studied the simultaneous effect of ultrasound and hypochlorite treatment on the selected properties of corn starch. Ozonated-ultrasonicated starches can be utilised in foods where lower viscosity at high solids content, better clarity, film forming and lower retrogradation properties are needed. Ozonated-ultrasonicated starches can also be useful in reducing processing times and energy consumption (Chang *et al.*, 2017; Chong *et al.*, 2013). To the best of our knowledge, there is no available research on the combined effect of ozonation and ultrasonication processes on starch properties. Therefore, the purpose of this research is to examine rheological and thermal properties of rice starch when ozonation and ultrasonification processes are applied in series on rice starch in aqueous form.

## 2. Materials and methods

### Rice starch

Native rice starch was obtained from commercial sources (Smart Chemistry, İzmir, Turkey). Starch was analysed for the ash and protein content as described in AOAC (1995). The moisture content of the starch samples was determined using a moisture analyser (MJ33 moisture analyser; Mettler Toledo, Greifensee, Switzerland). Rice starch used had 12.0 g water/100 g (db), 0.8 g protein/100 g (N×5.95) (db) and 0.2 g ash/100 g (db). The pH was 6.64. The starch was used directly without any purification.

### Ozonation process

An ozone generator working on the coronal-discharge principle was used to produce ozone gas (OMS, İzmir, Turkey). This generator was equipped with an integrated oxygen production unit which uses atmospheric air. The generator was able to produce ozone gas at a rate of 60 g ozone gas/h. Ozone gas from the generator was directed to a 500 ml glass bottle using a plastic pipe with a gas dispenser end to create bubbles for increased solubility of ozone gas in distilled water. Undissolved ozone gas was returned to the atmosphere using a discharging tube. Oxidation/reduction potential (ORP) method was used

to measure ozone concentration in aqueous solution. The ORP values in mV were converted to ozone concentration (g ozone gas/100 g solution) by means of a reference table provided by the manufacturer. Five grams of starch powder (dry basis) was added to 100 ml distilled water and the resulting solution was transferred to the 500-ml glass bottle. The contents of the glass bottle were ozonated for 1 h to have a concentration of 0.00042 g dissolved ozone/100 g water. The starch-water suspension was kept at 5-7 °C during ozonation by circulating cold water around the 500-ml bottle. Control samples were prepared by employing exactly the same processing steps. The flow rate of ozone gas from generator to the 500-ml glass bottle was kept at a rate 0.103 l/min.

### Ultrasonic treatment

Starch powder-distilled water solutions were prepared by adding five grams of starch (dry basis) to 100 ml of distilled water in a 250-ml bottle and this solution was ultrasonicated in power ultrasound range (16-100 kHz) for 10 min using an ultrasonic processor (Soniprep 150; MSE, London, UK) operating at a frequency of 16 kHz and an amplitude of 100%. The probe used was working with a transformation ratio of 3.8/1 and had a tip diameter of 9.5 mm. The temperature of the starch-water suspension was kept constant at 20 °C during ultrasonication by circulating cold water around the 250-ml bottle controlled with a thermostat. Control samples were prepared by applying exactly the same processing steps as ultrasonicated ones without the ultrasonication process.

### Combination of ozonation and ultrasonication processes

Starch solutions were subjected to a combination of ozonation and ultrasonication treatments. For this purpose, solutions were ozonated first as described above and ultrasonication was applied to ozonated solutions immediately. In order to see the effect of order of the combination, another solution was ultrasonicated firstly and then ozonation was applied without any delay.

### Rheological properties

Native (control), ozonated, ultrasonicated, ultrasonicated-ozonated and ozonated-ultrasonicated rice starch solutions, having concentration of 5 g starch powder/100 ml distilled water, were gelatinised by boiling at 95 °C for 10 minutes by gently stirring. The starch pastes obtained were then allowed to cool to room temperature. The viscosity of starch pastes were measured at 25 °C using a CVOR Bohlin Rheometer (Malvern Instruments Ltd, Malvern, UK) equipped with parallel plate geometry (20 mm diameter, 1 mm gap). 1 ml sample was carefully placed over the plateau of the rheometer. Once the plateau had been in contact with the plate, the surface of the sample was covered

with a thin layer of low-density silicone oil for preventing possible evaporation during the measurement. Viscosity values were calculated by dividing shear stress by shear rate. The data were fitted to the well-known power law model (Equation 1) to evaluate the variation in the flow properties of samples under steady shear (Choi and Yoo, 2009):

$$\tau = K\gamma^n \quad (1)$$

where,  $\tau$  = the shear stress (Pa),  $\gamma$  = the shear rate (1/s),  $K$  = the consistency index (Pas<sup>n</sup>), and  $n$  = the flow behaviour index (dimensionless).

### Differential scanning calorimeter measurements

A Perkin-Elmer differential scanning calorimeter (DSC 6; Perkin-Elmer, Norwalk, CT, USA) was used to determine the gelatinisation properties of the samples. The sensitivity of the DSC used was 0.5 mcal/s and it was calibrated with indium. Sample suspensions corresponding to exactly 30 mg starch sample (db) were placed into steel DSC pans and hermetical sealing was then applied. The sealed pans were allowed to equilibrate for 1 h before analysis. The samples were heated from 30 to 150 °C at a rate of 5 °C/min with a nitrogen flushing rate of 40 ml/min. A sealed empty pan was used as a reference. The DSC data was used to calculate onset, peak and conclusion temperatures during gelatinisation of samples, together with gelatinisation enthalpy ( $\Delta H$ ).

### Statistical analyses

The data was statistically evaluated for analysis of variance (ANOVA), Duncan's multiple comparison test and coefficient of variations using SPSS (SPSS, Inc., Chicago, IL, USA) with  $P < 0.05$  level. Triplicate replications were done.

## 3. Results and discussion

### Rheological properties and viscosity changes

The power law model was used to evaluate the flow characteristics of native and processed rice starch samples. Table 1 shows the flow behaviour ( $n$ ) and consistency indices ( $K$ ) for rice starch samples treated under different conditions. It was observed that all starch samples including the native starch exhibited non-Newtonian behaviour as indicated by  $n$  values smaller than one (Table 1).

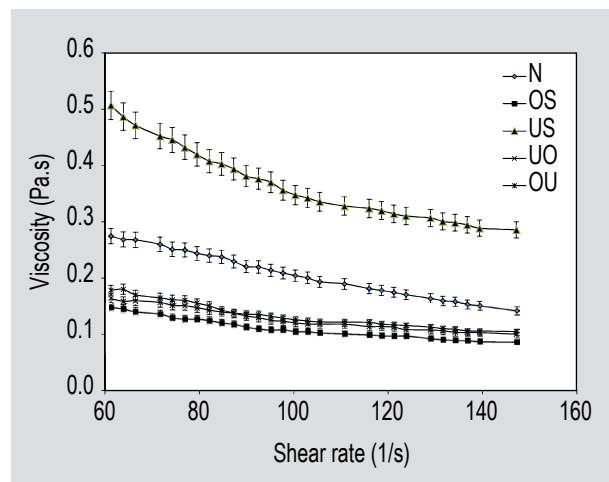
The highest viscosity values and the highest shear-thinning effect were observed for ultrasonicated starch sample (Figure 1, sample U).

The  $n$  and  $K$  values of all samples are significantly different from each other except the UO and OU samples which are having the similar flow properties (Table 1, Figure 1).

**Table 1. Flow properties (flow behaviour index ( $n$ ), dimensionless; consistency index ( $K$ ), Pas<sup>n</sup>) of native (N), ozonated (O), ultrasonicated (U), ozonated-ultrasonicated (OU) and ultrasonicated-ozonated (UO) rice starch samples.<sup>1</sup>**

Starch sample	$n$	$K$ (Pas <sup>n</sup> )	$R^2$
N	0.40±0.01a	6.10±0.01c	0.98
O	0.80±0.01c	0.45±0.01a	0.97
U	0.40±0.01a	12.01±0.01d	0.95
OU	0.75±0.1b	4.21±0.01b	0.98
UO	0.72±0.1b	4.12±0.01b	0.98

<sup>1</sup> Values followed by a different letter in the same column are significantly different ( $P < 0.05$ ). Means are based on triplicate analyses ( $\pm$  standard deviation).



**Figure 1. Changes in viscosity of rice starch with shear rate (N = native; OS = ozonated; US = ultrasonicated; UO = ultrasonicated-ozonated; OU = ozonated-ultrasonicated).**

The increase in  $K$  values can be explained with enhanced viscous properties of starch after ultrasonication (Azizi and Farahnaky, 2016). It is possible that shorter starch molecules come close to each other to form networks with increased viscous properties of ultrasonicated starch sample. The similar behaviours were exhibited by wheat and maize starch pastes reported in the literature for sonicated wheat and corn starch samples (Jambrak *et al.*, 2010). There was no significant difference between the consistency index of samples treated with ozonation and ultrasonication with no regards of treating sequence. This shows that the order of treatment (i.e. ozonation and ultrasonication) does not have any significant effect on the flow properties of rice starch studied under the conditions applied in this research. The viscosity changes in starch suspensions are due to possible partial depolymerisation of starch molecules upon ozonation (Chan *et al.*, 2011) and ultrasonication (Jambrak

*et al.*, 2010). As a result of ozonation and ultrasonication the starch chains may become shorter and low molecular weight dextrans may form. This may affect the consistency index and flow index of the samples directly. It was stated that ozonated starches may have lower viscosity values as a result of breakdown of starch molecules. Chemical group of molecules with higher level of carboxyl groups may cause lower viscosity values with incorporation of more water molecules into the structure (Sanchez-Rivera *et al.*, 2009). Ultrasonication creates shear forces, free radicals and increase in temperature; all leading to changes in starch structure (Zhu, 2015). Ozonation, on the other hand, may lead to two important reactions occur during the oxidation of starch. The first one is the oxidation of hydroxyl groups to carbonyl groups and the second one is the depolymerisation of starch molecules by cleavage of  $\alpha$ -(1 $\rightarrow$ 4)-glucosidic linkages (Vanier *et al.*, 2017). Therefore, the mechanism of structural and rheological changes in starch molecules during combined action of ozonation and ultrasonication seems complicated and requires further research, which is currently underway in our laboratory.

### Thermal properties

Gelatinisation properties of native and processed starch samples, as determined by DSC, are given in Table 2.

The onset gelatinisation of starches upon ozonation increased significantly as compared to native one with a significant decrease in gelatinisation enthalpy (Table 2, samples N and O). Similar results have been found for wheat and corn starches in our previous studies (Çatal and İbanoğlu, 2012, 2014). Oxidation by ozonation may result in the cleavage of starch molecules with lower molecular weight (Chan *et al.*, 2012). It is reported that small starch molecules would have higher gelatinisation temperatures (Alvani *et al.*, 2011) due to possible reorganising of small molecules upon heating with a higher temperature demand. However, the gelatinisation enthalpy of ozonated sample has been decreased when compared with the native one (Table 2). Although requiring higher gelatinisation temperatures, the whole gelatinisation process may be completed in a

shorter time, giving smaller enthalpy values. This can be attributed to the decreased crystalline structure of granules. Chan *et al.* (2011) found that the gelatinisation properties of corn, sago and tapioca starch did not change significantly after exposing dry starch powders to ozone gas. The gelatinisation properties depend on granule size, amylose-amylopectin ratio and the extent of crystallinity. The increase in the gelatinisation temperatures and the decrease in gelatinisation enthalpy obtained in ozonated sample can be attributed to the aqueous phase application of ozone rather than exposing starch to gaseous ozone. It has been stated that extent of ozone effect in aqueous phase is greater when compared to a treatment in the gaseous phase (Tiwari *et al.*, 2010). Therefore, ozonation in the aqueous phase could result in more destruction of starch crystalline structure in our starch samples. The effect of ultrasonication on gelatinisation properties of rice starch is given in Table 2. There is a slight but statistically significant decrease in the onset temperature of gelatinisation upon ultrasonication while the enthalpy of gelatinisation increased significantly as compared to native rice starch sample. It was reported that ultrasonication reduced the onset temperature and enthalpy of the gelatinisation of non-waxy rice starch while taro starch exhibited a slight decrease in onset gelatinisation temperature and an increase in the enthalpy value with the ultrasound time (Yu *et al.*, 2013). Research made for corn starch showed that gelatinisation temperatures in DSC measurements shifted slightly to higher values due to the sonication process with an decreased enthalpy value. This was attributed to occurrence of amorphous areas destructed by ultrasonication (Amini *et al.*, 2015). Our results for ultrasonicated sample (Table 2, sample U) are in line with the results of Yu *et al.* (2013) in terms of onset temperature and enthalpy of gelatinisation.

In order to study the effect of order of ozonation and ultrasonication, two different starch samples were prepared with different order of treatment: (1) ozonation followed by ultrasonication (OU); and (2) ultrasonication followed by ozonation (UO). Results showed that changing the order of treatment did not cause any significant changes in the gelatinisation properties of the samples (i.e. UO and OU,

**Table 2. Gelatinisation properties of native (N), ozonated (O), ultrasonicated (U), ozonated-ultrasonicated (OU) and ultrasonicated-ozonated (UO) rice starch samples.<sup>1</sup>**

Starch sample	Onset temperature (°C)	Peak temperature (°C)	Conclusion temperature (°C)	Enthalpy of gelatinisation (J/g)
N	75.9±0.4c	79.8±0.3b	84.5±0.2b	6.0±0.3c
O	76.8±0.1d	81.5±0.4c	85.4±0.1b	5.2±0.2b
U	74.9±0.3b	81.2±0.4c	85.9±0.1b	9.6±0.2d
OU	72.3±0.1a	76.1±0.3a	81.2±0.2a	4.5±0.3a
UO	72.5±0.2a	75.9±0.3a	81.5±0.3a	4.6±0.1a

<sup>1</sup> Values followed by a different letter in the same column are significantly different ( $P < 0.05$ ). Means are based on triplicate analyses ( $\pm$  standard deviation).

Table 2.) This suggests that order of treatment does not affect the gelatinisation properties significantly. However, these gelatinisation properties were significantly different than native, ozonated and ultrasonicated samples (i.e. N, O and U, Table 2). The combined effect of ozonation and ultrasonication is leading to starch samples with lower gelatinisation temperature and lower gelatinisation enthalpy requirement. This is a desired situation where lower energy is needed for starch gelatinisation. It seems that if there is a need for starch products with low gelatinisation temperature and low enthalpy values, rather than processing with ozonation or ultrasonication on their own, a two-step processing is more advantageous from energy requirement point of view. Ozonation generally leads to partial degradation of starch molecules leading to lower energies for gelatinisation compared to native starch (An and King, 2009). It is also stated that ultrasonication destroys the functional groups of starch molecules thereby an increase in gelatinisation enthalpy (Zhu, 2015).

#### 4. Conclusions and future work

The highest viscosity values and the highest shear-thinning effect were observed for ultrasonicated starch sample. The order of treatment (i.e. ozonation and ultrasonication) does not have any significant effect on the flow properties of rice starch. Results showed that switching the order of treatments did not cause any significant changes in the gelatinisation properties of the samples. It can be suggested that the mechanism of structural changes in rice starch molecules during combined action of ozonation and ultrasonication should be investigated vigorously in future research. By this way, it would be possible to estimate the behaviour of starch molecules when processed by ozonation and ultrasonication simultaneously.

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