CHAPTER 9

EXOPOLYSACCHARIDE IN FOOD INDUSTRY: PREVENTION OF SYNERESIS
9.1. Introduction

Starch is one of the most abundant and widely distributed components in foodstuffs such as bread and noodles. Commercial starches are obtained from seeds, particularly corn, waxy corn, high amylose corn, wheat and various rices and also from tuber or roots of potato, sweet potato and cassava. Its gelatinization is commonly achieved by cooking in the presence of water. On cooling, starch granules recrystallize to form a solid gel, a process known as retrogradation. A decrease in temperature causes a reduction in the kinetic energy that facilitates the amylose molecules to associate and form a three-dimensional network. As a consequence, water is squeezed out of the gel, a process generally referred as syneresis (Fig. 9.1), while intermolecular interaction between amylose molecules becomes stronger and gel shrinks.

![Diagram showing hydrogen bonds formed during retrogradation and release of hydrogen bonded water](image)

**Fig. 9.1.** Syneresis of starch gel, showing release of water from amylose gel
Syneresis negatively affects the functional and sensory properties of foods (Zheng et al., 1998; Sikora et al., 2003). It should be minimized without interfering with the native properties of food products. This could be controlled by chemical modifications, but blending starch or milk products with polysaccharide hydrocolloids can function as an alternative to the expensive chemical modification (Appelqvist & Debet, 1997). Most polysaccharides used by the food industry as bio-thickeners are derived from plants such as starch, pectin, guar and seaweed (carrageenan, alginate). These are not always readily available and their rheological properties often do not match those required. Hence, most polysaccharides of plant origin require chemical modifications to improve their structure and rheological properties. The consequence of these chemical modifications is that the polysaccharides carry heavy restrictions over their usage in food products.

Interest in using microbial exopolysaccharides in food processing has been increased in recent years because of several reasons like their nature of polydispersity, water binding and low solution viscosity, their ease of production by fermentation and their ease of manipulation by recombinant DNA technology (Johns & Noor, 1991; Morris, 1995). Starch/hydrocolloid mixtures are widely used to modify and control the texture of foodstuffs. The addition of a hydrocolloid strongly influences the gelatinization and retro-gradation of starch. Specifically, food hydrocolloids are used to thicken gel, control syneresis, stabilize an emulsion or suspension, function as a coating and bind water. It has been demonstrated that the structure of the hydrocolloid, including the type and number of monosaccharide backbone as well as the type, number and distribution of side units, determines its characteristics and behaviour in solutions. Understanding such properties will lead to improvements in the formulation of starch-based foods. All these aspects had thrown light into the study of exopolysaccharides from Lactobacillus plantarum on prevention of syneresis in starch and their rheological characterization. Understanding this aspect emphasizes the efficiency of
Lactobacillus plantarum exopolysaccharide in syneresis reduction and it was proved a potential candidate replacing carboxy-methyl cellulose, to improve the texture of starch-containing foods by increasing the viscosity of the final product and also by holding the water released.

9.2. Materials and Methods

9.2.1. Raw Materials

The raw materials, wheat and cassava starches used for syneresis studies were obtained from Sd- fine chemicals, Mumbai and Central Tuber Crops Research Institute (CTCRI), Trivandrum.

9.2.2. Preparation of starch-exopolysaccharide dispersion

Starch powder was dispersed in distilled water and cooked for 15 min in a boiling water bath with gentle mixing until thickening of paste. The mixture was allowed to reach room temperature before being aliquoted. Polysaccharide dispersion was made separately. EPS was dispersed in distilled water and then stirred in a magnetic stirrer until complete dissolution. Starch suspension and EPS dispersion were mixed before being aliquoted. Sodium benzoate or sodium meta bisulphite was added to the mixture to prevent microbial activity (1 g/l).

9.2.3. Estimation of Syneresis

The extent of syneresis in the formulations was estimated according to the protocol mentioned by Viñarta et al (2006). The liquid phase length (Δh) separated above the sedimented phase was measured in two day interval time throughout the storage at 4 °C for 20 days. The degree of syneresis was represented by Δh/h₀, where h₀ stands for the initial height (in cm) of the sample dispersion.
9.2.4. Selection of Starch

Cassava starch and wheat starch were chosen at 2 % (w/v) concentration for the initial studies. Concentrations of the wheat starch experimented were 2, 3, 4, 5 & 6 % (w/v) and for the subsequent studies, 6 % (w/v) wheat starch was used since the degree of syneresis was higher in 6 % wheat starch and it was stable throughout the storage.

9.2.5. Comparison of EPS Efficacy with Carboxy Methyl Cellulose

The efficacy of EPS in prevention of syneresis was compared with a control, carboxy methyl cellulose (CMC, Sigma Aldrich, USA), a viscosifier currently in use in food industry. The concentrations of wheat starch chosen for the experiment were 2, 4 and 6 % (w/v) and the concentration of CMC and L. plantarum EPS chosen for the comparison studies was 0.2 % (w/v).

9.2.6. Prevention of Syneresis in Starch by exopolysaccharide

The ability of exopolysaccharides (EPS) produced by Lactobacillus plantarum, to minimize the liquid separation (syneresis) experienced by cooked starch pastes during refrigeration was evaluated. The starch samples or mixture (starch and polysaccharide) were poured into screw-cap plastic tubes to reach a final volume of 15 ml. Tubes were held vertically at 4 °C for 20 days, unless otherwise stated and the extent of syneresis was estimated as mentioned above (section 9.2.3).

9.2.7. Rheological characterization of wheat starch-exopolysaccharide hydrocolloid

The rheological characterization of wheat starch, wheat starch-carboxy methyl cellulose and wheat starch-exopolysaccharide was studied using Paar Physica Moderate Compact Rheometer MCR 15 (Pongsawatmanit & Srijunthongsiri, 2008). Test samples were subjected to shear rate 0-100 s\(^{-1}\) at 25 °C. The apparent viscosity of the samples was
calculated at each point and the relationship between shear rate and shear stress was evaluated. The apparent viscosity was expressed in mPa s. The behaviour of wheat starch-exopolysaccharide at low and high concentrations of EPS was evaluated.

9.3. Results and Discussion

9.3.1. Selection of Starch

Starches of two different natures were selected for the study. Cassava starch is a tuber starch while wheat starch is a cereal grain type. Cassava starch is oval truncate in shape with an average size of 25 µm while the other is round lenticular of the same size. The tuber and root starches have a lower fat content than the grain starch. Out of the two different starches (2 % w/v) explored, the percentage of syneresis observed was more in wheat starch compared to cassava starch (Fig. 9.2).

![Fig. 9.2. Syneresis in wheat and cassava (2 % w/v) starches](image)

It has been discussed by Zheng et al (1998) that root and tuber starches exhibit high stability to cold storage. The result obtained indicated that the percentage of syneresis in wheat starch
has to be dealt with rather than the cassava starch. So wheat starch was selected for the
syneresis studies using exopolysaccharide from *L. plantarum*.

**9.3.2. Comparison of EPS Efficacy with Carboxy Methyl Cellulose**

Carboxy Methyl cellulose (CMC) or cellulose gum is used in food science as a
viscosity modifier or thickener and to stabilize emulsions in various products including ice-
cream. Taking into consideration this aspect, low viscosity CMC was used as a control to
compare with EPS from *L. plantarum* which is of low viscous nature at a concentration of
0.2 %. This particular concentration of the hydrocolloids was chosen for comparison, as a
minimum concentration. Usually, polymers exhibit significant influence in their flow
behaviour even from 0.2 % and hence are used at lower concentrations in food preparations
in the range of 0.2-1 %. Positive effects may be attained at levels as low as 0.1–1 % (Sikora
*et al.*, 2003; Gimeno *et al.*, 2004; Sadar, 2004). On comparison, the EPS from *L. plantarum*
was found to have a better efficacy than CMC in prevention of syneresis in cooked starch
pastes. The performance of *L. plantarum* EPS in all the three starch concentrations (2, 4 and
6 %) was impressive than CMC.

The percentage of syneresis inhibition in 6 % (w/v) starch with 0.2 % (w/v)
*L. plantarum* EPS was higher (30 %) than the other two on second day and observed a drop
on the fourth day onwards and reached 8 % on the 20th day on storage at 4 °C (Fig. 9.3).
Interestingly, in a similar formulation (6 % (w/v) starch with CMC, there was no syneresis
inhibition at all. But in the other two formulations, 2 and 4 %, the percentage of inhibition
increased up to 17 and 25 % with 0.2 % (w/v) *L. plantarum* EPS respectively and it was
almost stable throughout the storage. The percentage inhibition difference of CMC and
*L. plantarum* EPS in these two formulations was nearly 2-8 %. In other words, the
EPS from *L. plantarum* exhibited a 2-8 % higher efficiency than the commercially used
viscosifier, CMC.
The percentage inhibition of syneresis with *L. plantarum* was promising in all the formulations, but of varying degree. But the inhibition was not stable throughout storage at 4°C in 2% and 6% wheat starch formulations. The effect of both the hydrocolloids in 6% wheat starch was not satisfactory. Taking into consideration this aspect, 6% wheat starch was considered for the subsequent studies with *L. plantarum* EPS as CMC had no effective inhibition at the particular lower concentration.

**Fig. 9.3. Comparison of syneresis inhibition of CMC and *L. plantarum* EPS in different starch concentrations**

This experiment focuses the importance of *L. plantarum* EPS as a substitute of CMC in food preparations. CMC derived from bulky components, or pulp cellulose, of plant material, is chemically derivatized to make it water soluble while the EPS from *L. plantarum* is readily soluble in water. *L. plantarum* EPS performs effectively in the prevention of syneresis in addition to the role of a viscosifier.
9.3.3. Prevention of Syneresis in Starch by exopolysaccharide

As mentioned in the above section, prevention of syneresis was observed in all starch concentrations (2, 4 and 6 % w/v) with 0.2 % (w/v) *L. plantarum* EPS. Varying concentrations (0.2-1 % (w/v)) of *L. plantarum* EPS in 6 % (w/v) starch were found to be effective. Even though, inhibition in syneresis was observed with all concentrations, the rate of inhibition was around 50 % with 1 % EPS while it was 8 % with 0.2 % EPS (Fig. 9.4). All other concentrations displayed a percentage of inhibition between these values. With 0.4 %, EPS an inhibition of 9 % was obtained. The inhibition that could be attained with 0.6 and 0.8 % of EPS was almost same, 42 %.

EPS have the ability to bind water, which promotes the syneresis prevention on addition to cooked starch pastes. This was proved with the experiment that the incorporation of EPS in starch containing foods can reduce syneresis. Phase separation in aqueous solutions was observed at higher concentrations of *L. plantarum* EPS (Fig. 9.5), thereby bringing out an enhancement in the viscosity of the mixed system. EPS prevent syneresis and improve product stability firstly by increasing the viscosity and elasticity of the final product and secondly by binding hydration water. The importance of hydrocolloids in food industry has been discussed by many researchers as it is used to improve mouth-feel, texture, visual and taste perception, storage stability, mechanical protection and prevention of syneresis in the final food products (Broadbent *et al*., 2003; Hassan *et al*., 2003; Sikora *et al*., 2003).

9.3.4. Rheological characterization of wheat starch-exopolysaccharide hydrocolloid

The rheological behaviour of wheat starch, wheat starch-carboxy methyl cellulose and wheat starch-exopolysaccharide hydrocolloid was investigated by shear stress controlled rheometry. It is speculated that the increased viscosity of EPS-containing foods may increase the residence time of ingested fermented product in the gastrointestinal tract and therefore be
Fig. 9.4. Prevention of syneresis in wheat starch (WS, 6 % (w/v)) by *Lactobacillus plantarum* exopolysaccharide (EPS) at different concentrations is beneficial to a transient colonisation by probiotic bacteria. Fig. 9.6 displays the changes in the apparent viscosities of wheat starch-water, wheat starch-carboxy methyl

Fig. 9.5. Phase separation observed in wheat starch- EPS dispersion at higher concentrations
cellulose and wheat starch-exopolysaccharide (wheat starch-EPS) dispersion. It was found that the starch-EPS hydrocolloid displayed higher apparent viscosity than the one without EPS. The viscosity of the starch-EPS hydrocolloid was higher than the control (starch-carboxy methyl cellulose hydrocolloid) in same concentration. Increasing the concentration of EPS in the starch suspension increased the apparent viscosity of the mixture. The incorporation of the *L. plantarum* EPS hydrocolloid at a concentration of 0.2 % could increase the viscosity by 14 %. The viscosity of the starch-EPS hydrocolloid was higher than

![Fig. 9. 6. Shear-thinning behaviour of wheat starch (WS-water) suspension, wheat starch-carboxy methyl cellulose (WS-CMC), wheat starch-exopolysaccharide (WS-EPS) hydrocolloids](image)

In studies undertaken on starch/hydrocolloid blends, synergistic effects were observed that resulted in an increase in the viscosity of the mixtures compared with starch or
hydrocolloid alone (Sandstedt & Abbott, 1964). The synergistic increase in paste viscosity might be considered to be the result of at least two effects. First, there may be interaction between exudate from the granule (solubilized amylose and low-molecular-weight amyllopectins) and the hydrocolloid (EPS). Second, the addition of EPS would mean that the forces being exerted on the granules in the shear field are much larger than those encountered in starch-water suspensions of equal starch concentration. These increased forces should significantly affect granule breakdown and the amount of material exuded into the medium.

Fig. 9. 7. Apparent viscosity changes observed in wheat starch-EPS (0.2 %) and wheat starch-EPS (1 %)
The dispersions exhibited a non-Newtonian and pseudo-plastic behaviour. The relation between shear stress and shear rate was not linear, which is the characteristic of a non-Newtonian fluid. The dispersions exhibited a shear-thinning behaviour as the apparent viscosity showed an increase at lower shear rate and decrease at higher shear rate. The shear-thinning behaviour of the dispersions is more evident when both apparent viscosity and shear rate are plotted in logarithmic scale (Fig. 9.8). From the figure, it is evident that the dispersions obey the power-law. Power-law region, \textit{i.e.}, the straight line region, is the portion where the apparent viscosity is not at all constant. In this region, we can approximate the behaviour by:

\[
\ln \eta = a + b \ln \dot{\theta}
\]  

(1)

Finally by using the connection between apparent viscosity, shear stress and shear rate we write the equation as:

\[
\tau = K \dot{\theta}^n
\]  

(2)

where $\tau$ is the shear stress, $K$, the consistency coefficient, $\dot{\theta}$, shear rate and $n$, power-law index. The power-law index for the dispersions was observed in the range 0.6-0.9 \textit{i.e.}, <1.

Similar behaviour was observed for the wheat starch-exopolysaccharide dispersions in all the concentrations of exopolysaccharide. The application of exopolysaccharides in varying industrial areas is mainly due to their rheological properties that allow the formation of viscous solutions at low concentrations (0.05–1.0 \%) and stability over wide temperature, pH and ionic strength ranges (Kumar & Mody, 2009). Incorporation of hydrocolloids in dairy foods or beverages is a well-known strategy to provide viscosity, stability and water-holding
capacity. This strategy of incorporation of hydrocolloids to provide the above characteristics has been made use of in the present experiment. The success of EPS application mainly depends on its ability to bind to the water and its efficacy to increase the viscosity of the final product and these were successfully attained in this particular case. This thermal stable EPS from *Lactobacillus plantarum* isolate along with its ability to bind water and increase viscosity, find its role in food industry as it can withstand heat processing and can protect foodstuffs during storage and transportation.

**Fig. 9. 8. Pseudoplastic wheat starch-EPS dispersions obeying power-law**

**9.4. Conclusion**

The study revealed that the incorporation of exopolysaccharides in starchy foods can increase the viscosity of the food and thereby improve the appearance and texture properties
of the food. The dispersions exhibited a non-Newtonian and pseudo-plastic behaviour. Phase separation was observed in the dispersions when higher concentrations of \textit{L. plantarum} EPS was used and a shear-thinning behaviour was eminent as the apparent viscosity showed an increase at lower shear rate and decrease at higher shear rate. The impact of exopolysaccharide in the prevention of syneresis in starch throws light into its use as an ingredient in starch-containing foods for better texture and mouth-feel. The improvement of viscosity of the final product and prevention of syneresis makes the \textit{Lactobacillus plantarum} EPS an effective substitute of CMC as viscosifier in foods.