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Atul Anand Mishra
Assistant Professor, Department of Processing and Food Engineering, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, India

RN Shukla
Assistant Professor, Department of Processing and Food Engineering, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, India

Corresponding Author:
Atul Anand Mishra
Assistant Professor, Department of Processing and Food Engineering, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, Uttar Pradesh, India

Effect of bromelain enzyme concentration to reduce steep time and SO_2 requirements in a enzymatic corn wet-milling process

Atul Anand Mishra and RN Shukla

Abstract
A very small scale laboratory procedure (≈20 g) is needed to test wet-milling characteristics of corn when amounts of corn available for testing are quite limited. The objective of this study was to downscale 10-g and 100-g laboratory wet-milling methods already widely used to measure wet-milling properties of 20-g of corn. A Standard 10-g and 100-g procedure, a Modified 100-g and 10-g procedure, and an Experimental 20-g procedure were compared using six corn verities with known differences in enzymatic wet-milling (enzyme concentration 0.5%, 1%, 1.5% & 2%) and steeping time (15 Hrs, 20 Hrs, 25 Hrs & 30 Hrs) properties also. Germ and fibre separation was conducted differently for each procedure and probably accounts for these differences when decanting methods was used. Enzyme Concentration and steeping time effects on the starch/gluten separation were more pronounced when the Experimental 20-g procedure was used, which may allow for more discrimination among corn. Although most fraction yields are too small to run replicates for analytical tests, the Experimental 20-g procedure will be useful in measuring milling efficiency of early generations of hybrids corn where limited samples are available, such as when valuable recombinant proteins are expressed for therapeutics and industrial enzymes.

Keywords: Enzymatic wet milling, steeping time, enzyme concentration, starch recovery

Introduction
An effective enzymatic process significantly reduced steeping time in a corn wet-milling process was reported. This was also the first report to demonstrate that the use of a protease alone, without other enzymes or sulfur dioxide addition, was sufficient to reach starch yields equivalent to or exceeding that of the conventional wet sulfite-milling process, other enzyme steeping processes have been developed that nominally decreased the required steeping time or to some extent improved starch recoveries. However, in these studies, enzymes were used in combination with high levels of SO_2, and no specific class of enzyme or enzymes responsible for improvements were identified.

Using reproducible laboratory fractionation procedures for conventional wet milling (Eckhoff *et al*. 1999) [8] the starch yields from adequately steeped corn are very close to the retical yields. This makes the differentiation of conventional yields and the potential improvements from an added enzymatic effect difficult, if not impossible, to distinguish statistically, even with the most reliable and reproducible methods. These hindrances, in combination with the diffusion limitations of using intact kernels, effectively prevented researchers from seeing the true potential for enzymes in grain fractionation processes.

There are certain classes of enzymes that can actually be activated by sulfur dioxide for other reducing agents) and others that retain activity at relatively high sulfite levels Cysteine pro teases, including bromelain, are in this category. Bromelain is an enzyme preparation extracted from the leaves of the pineapple plant and contains at least four distinct Cysteine proteases. Enzymatic milling (E - Milling) is a process that incorporates a short soaking step (4-30.hr) followed by a coarse grind and incubation under controlled conditions with a protease to release the Starch - protein interactions before traditional fractionation. E - Milling does not require sulfur dioxide for effective product, separation and has the potential to replace the sulfur dioxide procedure currently used in commercial wet - milling facilities. To be used in a continuous commercial operation, an effective microbial control agent would be required in the process The use of low levels of sulfur dioxide (sufficient for antimicrobial activity) is being proposed as an easily implemented procedure for microbial control during E - Milling while still maintaining the beneficial aspects of the process.

This research was undertaken to analyse the Effect of Bromelain enzyme concentration to Reduce Steep Time and SO_2 Requirements in a Enzymatic Corn Wet-Milling Process.

Materials and Methods

The six verities of Corn (JM-8, JM-12, JM-215, JM-216, and JM-218 & PJHM-1) were taken. Protease enzymes (bromelain from pineapple stem; were purchased from online market. Corn samples were hand-cleaned to remove broken kernels and foreign materials. Samples were then packaged and stored at 4 x ℃ until used.

Enzymatic Activity Measurements Protein content was determined according to Kjeldahl Method (AACC, 1983)

Wet-Milling Procedures Conventional corn wet-milling was done using the 100-g laboratory corn wet-milling procedure of Eckhoff *et al*. (1999) [8]. The twostage modified steeping procedure was conducted as follows. Samples of corn (20 g) were placed in 250-mL conical flasks with 60 mL of water or steeping chemicals (0.2% SO_2 + 0.55% lactic acid) and different concentration of bromelain enzyme (0.5%, 1%, 1.5% & 2%). The corn was soaked for 15 hr, 20 hr, 25 hr &30 hr with different concentration (0.5%, 1%, 1.5% & 2%) of bromelain concentration at 50 ℃. The water was drained into a 250-mL graduated cylinder and this unabsorbed water volume was measured and then dried to determine total solids using the two-stage drying procedure (Approved Method 44-18, AACC 2000). The diluted sample of the corn was washed for 4 -5 times to get the purified fractions of corn. The different sieves were used for separation of corn fraction. The germ was collected first having higher particle size secondly the fibre was collected in two sieve attached together having lesser particle size than the germ. Muslin cloth was also used for proper separation of Starch from the fibre. Lastly Starch was settled down in bottom pan having lesser particle size than fibre and germ respectively. Decanting process required 320 to 340 ml of distilled water for separation of all fraction of corn. The excess water was drained out from the collected starch. The starch was then placed in the Tray dryer and in oven for determination of moisture content and removal of moisture permissible layer.

Result and Discussion

Effect of Steeping Time (15, 20, 25 and 3 hrs) on Starch Recovery

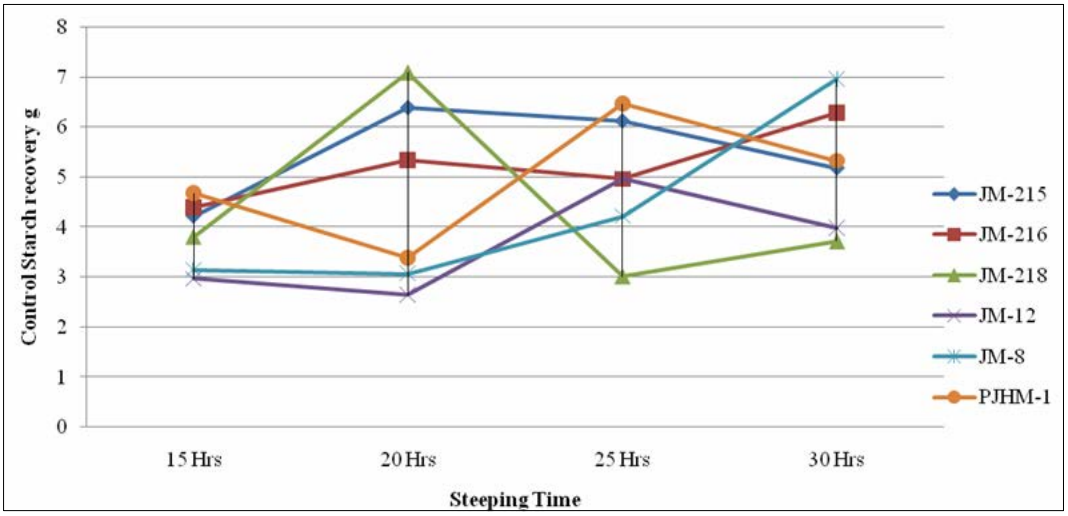

Fig 1: Effect of Steeping Time (15, 20, 25 and 3 hrs) on Starch Recovery

From above fig 1 it was clearly observed that maximum amount of starch was obtained in JM- 218 variety for 20 hours of steeping, i.e- 7.09 gm and minimum amount of starch was obtained in JM-12 variety for 20 hours of steeping, i.e- 2.64 gm.

Effect of Steeping Time (15, 20, 25 and 30hrs) on Starch Recovery at constant enzyme concentration 0.5%

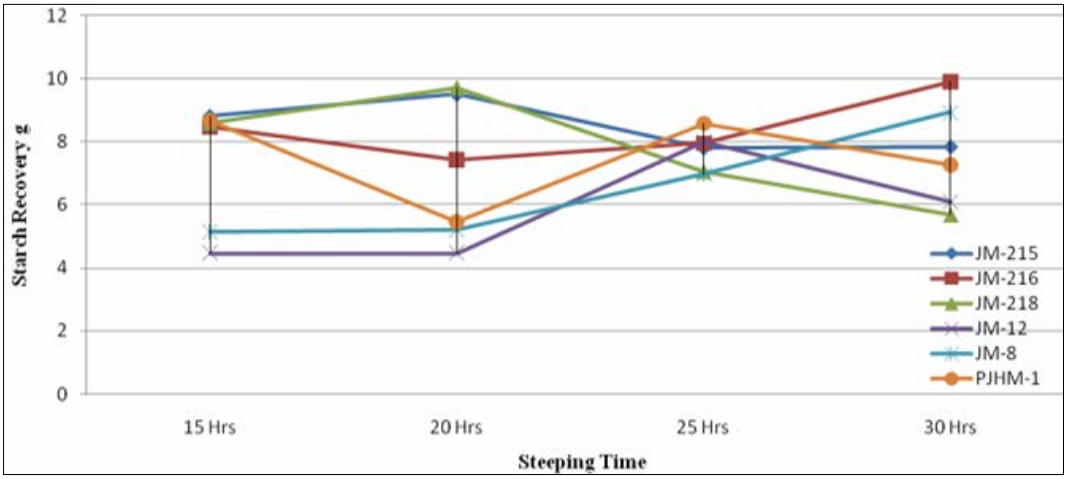

Fig 2: Effect of Steeping Time (15, 20, 25 and 30hrs) on Starch Recovery at constant enzyme concentration 0.5%

From above fig. 2 it is clearly observed that maximum amount of starch was obtained in JM-216 variety at constant enzyme concentration 0.5% for 30 hours of steeping, i.e.- 9.90 gm and minimum amount of starch was obtained in JM-12 variety at constant enzyme concentration 0.5% for 15 hours of steeping, i.e.- 4.46 gm.

Effect of Steeping Time (15, 20, 25, and 30hrs) on Starch Recovery at constant enzyme concentration 1.5%

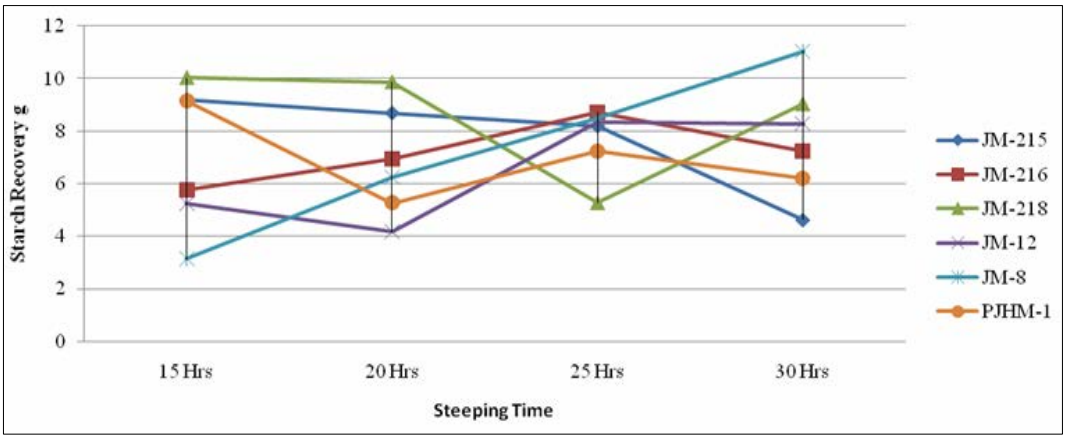

Fig 3: Effect of Steeping Time (15, 20, 25, and 30hrs) on Starch Recovery at constant enzyme concentration 1.5%

From above fig.3 it is clearly observed that maximum amount of starch was obtained in JM-8 variety at constant enzyme concentration 1.5% for 30 hours of steeping, i.e.- 11.04 gm and minimum amount of starch was obtained in JM-8 variety at constant enzyme concentration 1.5% for 15 hours of steeping, i.e.- 3.14 gm.

Effect of Steeping Time (15, 20, 25, and 30hrs) on Starch Recovery at constant enzyme concentration 2%

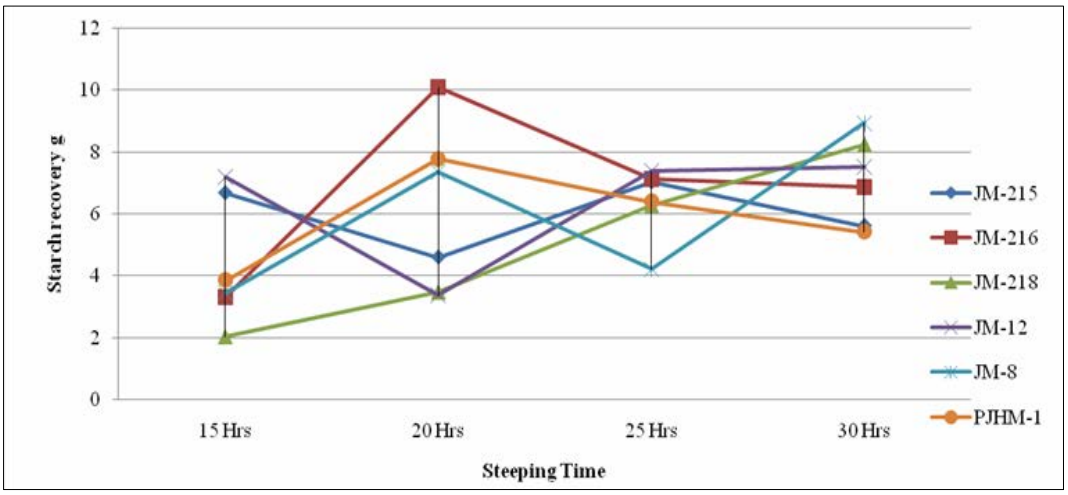

Fig 4: Effect of Steeping Time (15, 20, 25, and 30hrs) on Starch Recovery at constant enzyme concentration 2%

From Fig. 4 it is clearly observed that maximum amount of starch was obtained in JM-216 variety at constant enzyme concentration 2% for 20 hours of steeping, i.e.- 10.09 gm and minimum amount of starch was obtained in JM-218 variety at constant enzyme concentration 2% for 15 hours of steeping, i.e.- 2.03 gm.

From the above figures it is observed that maximum amount of starch was obtained in JM-8 variety at constant enzyme concentration 1.5% for 30 hours of steeping, i.e.- 11.04 gm and minimum amount of starch was obtained in JM-218 variety at constant enzyme concentration 2% for 15 hours of steeping, i.e.- 2.03 gm.

It was observed that starch yield was increases when the amount of enzyme increases. The addition of enzyme mainly reduced the amount of SO_2. Combining the reduced SO_2 with enzyme treatment helped decrease the enzyme dose without sacrificing starch yield. The effect of enzyme and SO_2 on increasing starch yield is synergistic.

Longer steep time allows soluble from the germ and endosperm to leach out into the steep water. Lower germ yields for enzymatic treatments were likely due to shorter soak times, leading to heavier germ (due to reduced leaching of water soluble proteins from germ) and reduced flotation (germs are recovered by flotation). Fiber yield decreases as the amount of enzyme is increased.

If the enzyme treatment in excess of 2%, then the enzyme had no further effect on reducing the fiber yields. With no enzyme or SO_2 addition during wet milling, a higher amount of starch lost in the fiber fraction.

So, depending upon the amount of enzyme and SO_2 an approximately increase in starch yield was observed for the enzymatic process compared to the conventional corn wet milling process. At the commercial level, the possible reasons for the larger increase in starch yield (in commercial trial compared to laboratory trial) could be the recycle effect of enzyme in the process water. In the commercial plant, process water from starch washing moves back into the process and ends up as steep water. It is possible and likely that some enzyme activity is left in the process water, which could reduce the enzyme requiring during steeping. Enzyme addition means the increase of proteolytic activity. Bromelain was selected for increasing starch yield and minimize the amount of enzyme necessary to maintain starch yield. Enzyme treatment with SO_2 and lactic acid has the better effect to separate the starch from the cell wall protein matrix. This approach removes the diffusion barriers and allows the enzymes to react with the endosperm associated proteins that encapsulate the starch granules and loosen the starch protein interactions. That's why the maximum amount of starch is obtained by reducing steeping time with addition of enzyme as compared to traditional wet milling.

Conclusion

This study confirms that the application of enzyme to the normal steeping process of wet milling is an effective mean of decreasing the steeping time or SO_2 uses. It's lightly that enzyme adequately penetrates the intact kernel and therefore degrades the starch associate protein.

This study demonstrated that when the enzyme was added to the ground corn are they effective in decreasing the steeping time. This approach removes the diffusion barrier and allows the enzyme to react with endosperm associated protein that in capsulate the starch granule and losing the starch protein interaction. The modified enzymatic wet milling process substantially reduces or eliminates the addition of the SO_2 while maintaining high product recovery. The soaking step must be sufficient before any amount of grinding, can be done otherwise germ yield and quality will be decrease. This was determined to be for at least 15hrs at 50 °C but could be longer. Soaking for up to 20 hrs did not adversely affect the starch recovery. The grinding step must sufficiently disrupt the endosperm to allow the enzyme penetration and hydrolysis to occur during the enzyme incubation step.

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