Structural Modifications of Gluten Proteins in Intermediate wheatgrass (*Thinopyrum intermedium)* Dough: Role of Dough Conditioner and Refinement Level

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INTRODUCTION

- *Thinopyrum intermedium*, commonly known as intermediate wheatgrass (IWG) is a novel perennial crop, with both environmental and nutritional benefits (*1* and *2*).
- IWG is currently mainly used as a forage but shows great potential to be developed as a grain crop.
- IWG has higher protein, fiber and antioxidant contents than that of hard red winter wheat (*3*).
- IWG is mainly consisting of gliadins and low molecular weight glutenins (LMWG) and deficient in high molecular weight glutenins (HMWG) suggesting a poor gluten forming ability (*4* and *5*).
- The difference in protein distribution coupled with higher fiber content negatively affects the protein network formation in the dough.
- It has been shown that competitive water binding by bran cause redistribution of moisture in wheat dough resulting in partial dehydration of gluten, which in turn causes conformational changes in gluten and adversely affect its viscoelastic properties (*6-7*).
- Protein secondary structure, and its stability, greatly impact gluten network strength.
- Dough prepared from partially refined IWG flour had a better protein secondary structure as profile compared to whole and fully refined flour, suggesting a good compromise between dough extensibility and elasticity. However, dough mixing parameters and protein structural elements revealed poor dough rheology.
- Therefore, the objective of this study was to employ dough conditioners and determine the effect of their presence along with bran reduction on the protein secondary structure using attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy.

MATERIALS AND METHODS

- **Materials**: IWG with the same genetic make-up, cultivated in 2015, in two Minnesota locations, Rosemount and Roseau were kindly provided by Department of Agronomy/ Plant Genetics, University of Minnesota. Control wheat (W) was provided by Grain Millers, Inc., Minnesota. The five dough conditioners used in the study were Ascorbic Acid (AA), Vital Wheat Gluten (VWG), Arise 8000; a wheat proten isolate (A), Powerbake, a commercial baking enzyme-xylanse (PB) and Transglutaminase (TG).
- **Milling of Grains**: IWG and HRWW grains were milled by a Brabender Quadrumat Junior mill (C.W. Brabender Instruments, Hackensack, NJ) according to AACC 26-50.01. Bran was further milled using a cyclone sample mill (UDY, Fort Collins, CO) equipped with a 0.25 mm screen.
- **Preparation of Flour Samples**: Bran was added back to refined flour at 0%, 50% and 100% of original bran content.
- **Dough Preparation**: Dough prepared to target moisture of 46.5% in a Kitchen aid Mixer. Mixing speed 2 for 2 min followed by 4 for 2 min.
- **ATR-FTIR Spectroscopy**: The infrared spectra of flour and dough samples were recorded using an ATR-FTIR spectrophotometer (Bruker Tensor 37, Bruker Optics, Inc., Billerica, MA, USA) equipped with a horizontal multi-reflectance zinc selenide crystal accessory (*7*).
- **Protein Secondary Structure Estimation**: The quantitative estimation of gluten secondary structure in dough was determined from second-derivative spectra of amide I region (1600 – 1700 cm^{-1}). The spectral regions were assigned as 1620-1644 for β-sheets, 1644-1652 cm^{-1} for random, 1652-1660 cm^{-1} for α-helix, and 1660-1685 cm^{-1} for β-turn structures. The second derivative area for each secondary structural region was divided by the total area of the amide I region (*8*) .

RESULTS

Effect of Dough Conditioner on Protein Secondary Structure

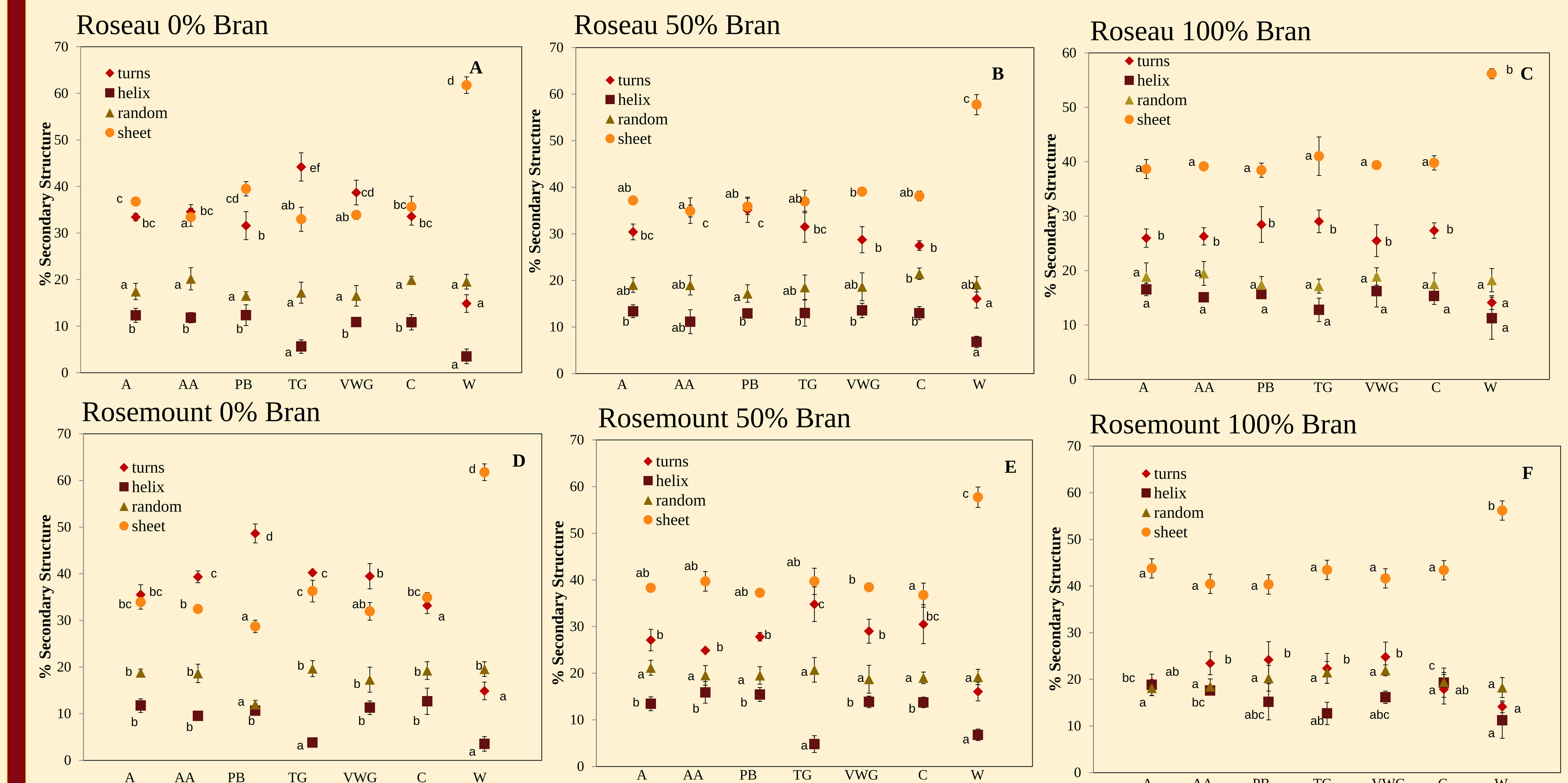

Figure 1: Effect of dough conditioner on protein secondary structure analyzed at growing location Roseau (A, B and C) and Rosemount (D, E and F). Different lower case letters indicate significant differences according to Tukey ($P \leq 0.05$) at each bran level and growing location.

Effect of Bran Level on Protein Secondary Structure

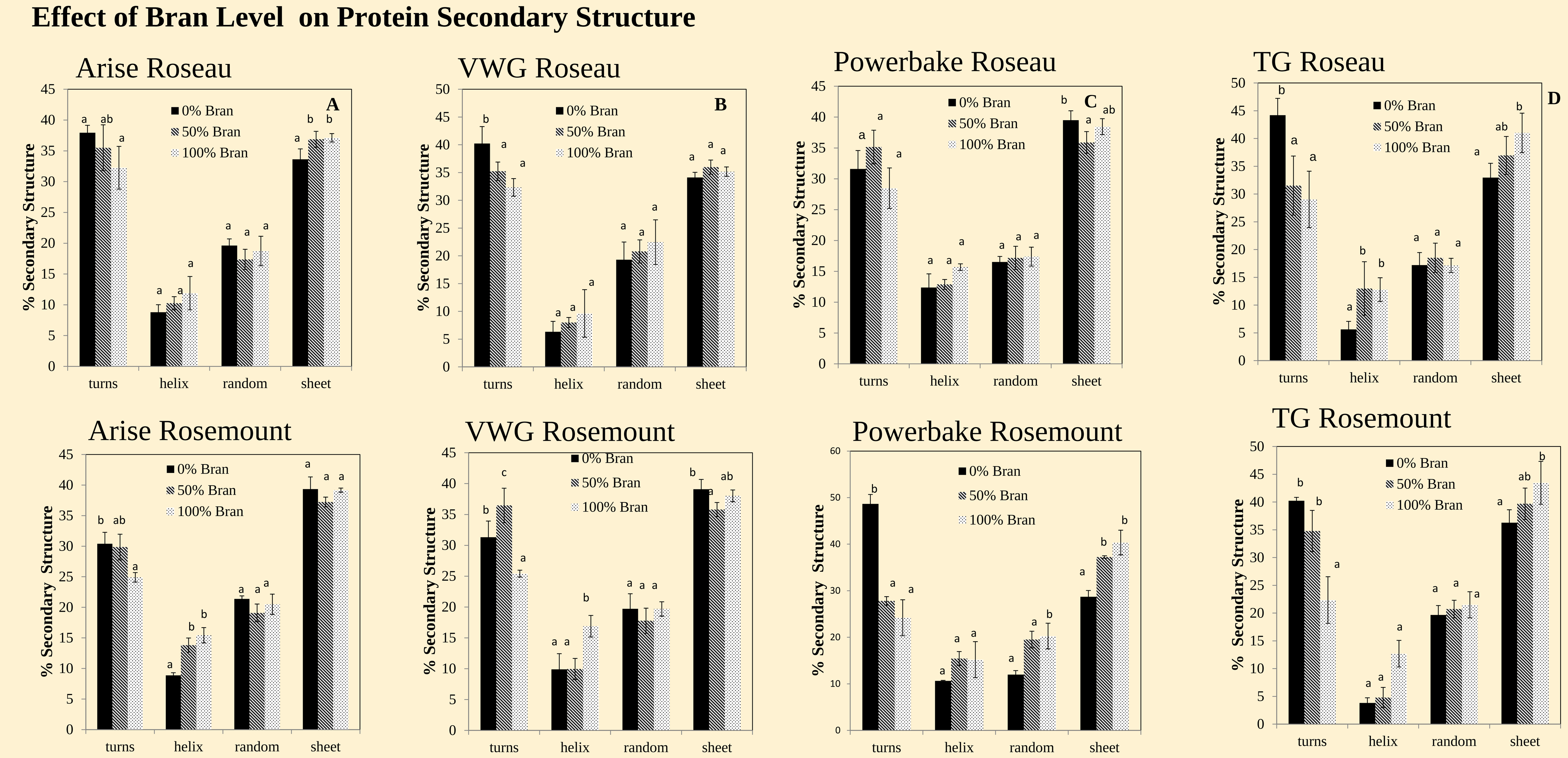

Figure 2: Effect of bran level on protein secondary structure analyzed at growing location, Roseau (top panel) and Rosemount (bottom panel) for Arise (A), VWG (B), Powerbake (C) and TG (D). Different lower case letters indicate significant differences according to Tukey ($P \leq 0.05$) for each dough conditioner and growing location.

Effect of Concentration of Dough Conditioner on Protein Secondary Structure

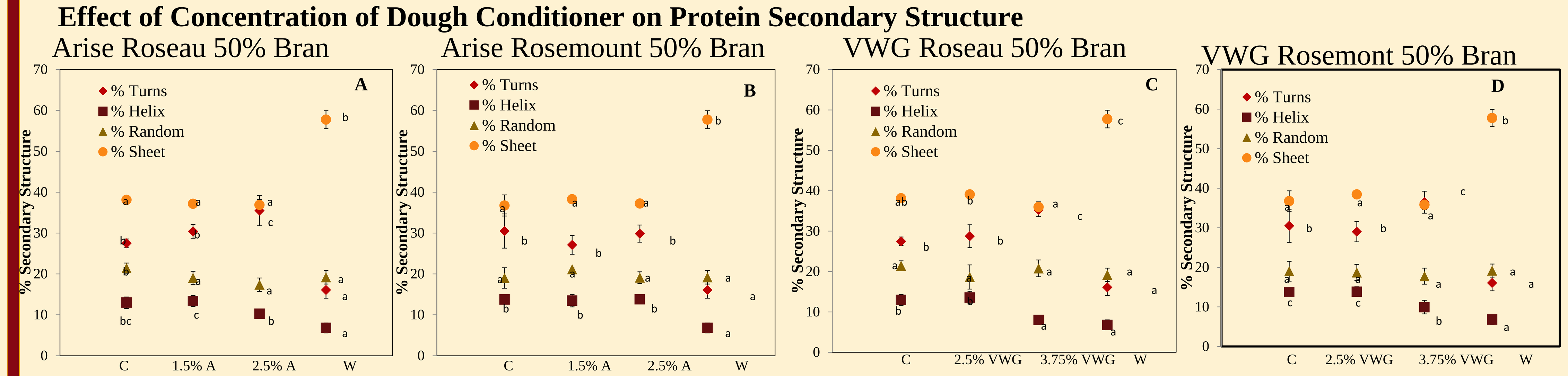

Figure 3: Effect of concentration of Arise (A and B) and VWG (C and D) on protein secondary structure analyzed at 50% bran level for both growing locations. Different lower case letters indicate significant differences according to Tukey ($P \leq 0.05$) for each dough conditioner and growing location.

DISCUSSION

- IWG showed a significantly ($P \leq 0.05$) lower amount of β-sheets content than that of wheat regardless of the refinement level and growing location (**Figure 1**) suggesting the lack of viscoelastic network formation in IWG dough.
- At 0% bran level, TG had a significantly ($P \leq 0.05$) higher amount of β-turns (**Figure 1A**) and a lower amount of α-helix (**Figure 1A and 1D**) than that of control (C: no dough conditioner). β-turns are important structures for proper gas holding capacity and loaf volume.
- The significantly ($P \leq 0.05$) lower amount of α-helix content seen in the presence of TG was equivalent to that of wheat.
- At 50% bran level, VWG Rosemount showed a significantly ($P \leq 0.05$) higher amount β-sheets at the cost of random structures than the control (**Figure 1E**) suggesting a stronger dough formation.
- The presence of dough conditioners did not significantly affect the secondary structure of IWG dough at 100% bran level (**Figure 1C and 1F**).
- As the bran level increased β-turns content increased with the gain of β-sheets or α-helix structures in the dough prepared with A and TG (**Figure 2A and 2D**) for both Roseau and Rosemount, VWG Roseau (**Figure 2B**) and PB Rosemount (**Figure 2C**).
- VWG Rosemount (**Figure 2B**) and PB Roseau (**Figure 2C)** showed a higher content of β-turns in the 50% bran level than that of 0% and 100% suggesting a good balance between gas holding capacity and dough elasticity.
- For 50% bran level, increasing the concentration of Arise for Roseau and VWG for Roseau and Rosemount, β-turns increased at the cost of α-helix structures (**Figure 3A, 3C and 3D**) with no significant changes for Arise Rosemount (**Figure 3B**).

CONCLUSION

- The dough conditioner, refinement level and growing location greatly affected the protein secondary structure elements in IWG.
- At 50% bran level, VWG and in refined flour, TG showed a better secondary structure profile compared to control IWG.
- Future studies should investigate the optimum concentration of TG and role of dough conditioners in bread making.

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