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ORIGINAL ARTICLE

Effect of Antifreeze Proteins on Protein Characteristcs and Modern State in Preferred Frozen Dough

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Abstract: The purpose of this study was to investigate the influence of antifreeze proteins (AFPs) on Protein PropertiesAnd the states of water in Frozen Dough During Frozen Storage and freeze-thaw cycles by using Ellman's colormetric method, sodium dodecyl sulfate-polyacryamide gel electrophoresis SDS-PAGE) fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). results shoe that the free sulphur content in frozen cough rose while the disulfide bond content created after frozen storage and freeze-thaw cycles. adding AFPs had no effect on the protein subunits. with increased frozen storage time, the content of internalBeta-Sheet created whereas the contentBeta-Turn structure dropped. freeze-thaw cycles created the contentAlpha-Helix structure. Frozen Dough with added AFPs shouwet smaller changes in all protein characteristics matched with the control sample with no added AFPs. The water loss rate in the control sample newly renovated; the relaxing timeT_{21}.Became greater with frozen storage time and the bound water content created. these findings illustrate that AFPs could inhibit the bond rust and changes in protein secondary structure, restrain the condensation of ice crystals, prevent water loss from the cough, and remain the water-holding capacity of the cough.

Keywords: Frozen cough; antifreeze proteins; Frozen Storage and freeze-thaw cycle; protein

With the flour products industry of rapid development frozen technology was more and more of application to dough production in Frozen Dough technology get rapid development[1]. But the study found that Frozen Dough steamed bread in Frozen Storage after up to less than fresh steamed bread of Quality[2]With the frozen storage time of extended Frozen Dough surface become rough finished product quality decreased[3]. Zhao Lei[4]Found when Gluten Protein frozen storage time more90 dAfter gluten network structure almost does not exist. Wang Pei[5]Study found frozen storage reduce the gluten protein and water of combined with role with the frozen storage time of extended Gluten Protein depolymerization to makes gluten network structure degradation. For this a kind of problem Liu piano and[6]The effect of frozen storage on the secondary structure of gluten protein was studied by circular dichroism.90 dSecondary Structure of late gluten The most significant changes.RibottaWait.[7]Using Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (Sodium Dodecyl sulphur-polyacrylamide gel

Electrophoresis,SDS-PAGE) It was found that the High Molecular Weight Gluten bands in Frozen Dough were weakened by freezing storage. It was believed that the understanding and aggregation behavior of gluten might occur due to recrystallization of ice crystals.

The fluctuation of temperature during freezing storage will cause recrystallization, destroy the gluten structure, and decrease the quality of Frozen Dough.[8-9]. In the process of Frozen Dough, with the extension of frozen storage time, the surface moisture of Frozen Dough dries up, and the internal moisture slowly moves to the surface, resulting in the loss of moisture in the dough.[10]With the extension of time, the reduction of moisture caused cracks on the surface, affecting the quality and appearance of Frozen Dough. When the temperature fluctuates, the ice crystals in the Frozen Dough continuously appear to melt and reorganize, and recrystallization occurs. The ice crystals generated by recrystallization are usually larger, which will destroy the gluten network structure, thus affecting the quality of Frozen Dough and finished products.[11-12]. Therefore, it is urgent to study how to improve the quality of Frozen Dough and realize the industrialization of traditional flour products.

At present, the food industry to improve the quality of Frozen Dough and Extend Shelf Life mainly rely on additives, antifreeze protein (Antifreeze proteins,AFPs) Is China's2006Annual approval of new additives for frozen food[13]. DomesticAFPsAlthough there have been many researches, most of them focus on bread production, and there is little research on the application of them in the traditional Chinese cooking and frozen noodle products. Du Haoran *et al*[14-15]Response surface methodology was used to optimize the process formula of Frozen Dough steamed bread and the compound additive formula of mixed starter for Frozen Dough steamed bread.DanielWait.[16]WillAFPsThe results showed that the quality of frozen dessert was significantly improved.PayneWait.[1 7]The study found that beef was soaked inAPFsIn solution, in-20 yenAfter freezing, the ice crystal volume is significantly reduced.Zhang ChaoWait.[18]StudiedAFPsEffect on texture characteristics of Frozen Dough.

AFPsIt can maintain the bread volume and improve the softness and hardness of the dough, which is mainly due to the decrease of the ice water content.

The purpose of this study is to investigate the frozen storage and freeze-thaw cycleAFPsThe Frozen Dough protein characteristics, water state and of influence revealAFPsIn Frozen Dough frozen storage and temperature fluctuation conditions under stable Frozen Dough quality of role effect and internal mechanism. ForAFPsImprove traditional Chinese cooking Frozen Dough products quality provide certain theory basis.

1.1 Material and reagent

High Gluten Wheat Flour Zhengzhou jinyuan flour factory; high activity dry yeast Angel Yeast Co., limited the company;AFPsZhengzhou givaudan Chemical Products Limited the company.

1.2 Instrument and Equipment

B 5Type Multi-Function mixer Guangzhou wws industrial limited the company;FX-15SBread fermentation tank Guangzhou sai si da Mechanical Equipment limited the company;HDGDJ-150High and Low Temperature alternating test chamber Shanghai, heng ding instrument equipment factory;LGJ-10DFrozen dryer, Beijing, four-ring Science instrument factory limited the company;UV-2000UV-Visible spectrophotometer, gel imaging system you ni ke (Shanghai) instrument limited the company;DYY-5Style regulated steady flow Electrophoresis apparatus, Beijing, six one instrument factory;Spectrum GXFourier

Transform Infrared Spectrum (Fourier transform infrared spectroscopyFTIR) Instrument AmericanPerkin ElmerThe company;PQ001 micromrCabinet nuclear magnetic resonance (Nuclear Magnetic ResonanceNMR) Imager Shanghai niumag electronic science and technology limited the company.

1.3 Methods

1.3.1 Frozen Dough and Frozen Dough steamed bread of preparation

100 gFlour and0.5 g AFPsMixed uniform (does not containAFPsOf

For blank dough); will mixed the flour into the dough mixer join1 gPre-

Activation of yeast,50 gWater and surface to no corn flour inclusion. Remove static10 minWill static the good dough with pressing machine pressure surface5Times (5 mm) Then segmentation30 gOf dough plastic after into Temperature35 ℃, Relative humidity85%Of proofing box in FermentationNatural 20 min. Stay dough temperature decreased after-40℃ Low temperature test chamber in quick-frozen30 minOut-18℃ Refrigerator in frozen storage.

Preparation Frozen Dough steamed bread when Will Frozen Dough placed Temperature35 ℃ , Relative humidity85%Of proofing box in thawing Fermentation30 min.

1.3.2 Frozen Dough Frozen Storage freeze-thaw of Processing

Will preparation good Frozen Dough in-18 ℃ Conditions under respectively Frozen Storage1,7/,15,30,60,90 dOut processing get different frozen storage time of sample to be measured.

Freeze-thaw cycle processing reference leaf actively and[19]Of methods slightly modify will Frozen Storage3 dOf Frozen Dough out in35 ℃ Constant Temperature in thawing30 minOut part dough processing remember for the first0Times freeze-thaw cycle; the rest of the dough-18 ℃ Refrigerator in Frozen Storage1 dAfter out by last time thawing conditions thaw out part dough processing remember for the first1Times freeze-thaw cycle; the rest part according to the above methods respectively the first2,3,4,5Times freeze-thaw cycle.

Samples by frozen storage freeze-thaw processing after out part the Frozen Dry crush.120Mesh sieve Mount bag in standby.

1.3.3 Frozen Dough free thiol and two sulfur key content of Determination

Said take75 mgCrush Sieving of Frozen Dough samplesEllman'sReagent Colorimetric Method Determination Frozen Dough two sulfur key and free thiol of content[20].

1.3.4 Frozen DoughSDS-PAGEDetermination

Reference Wang Jing[21]The methods the one-waySDS-PAGEAnalysis Frozen Dough in polymer quality glutenin subunit (High Molecular Weight-glutenin

ApplHMW-GS) And low molecular quality Glutenin Protein subunit (Low Molecular Weight-glutenin applLMW-GS) Component of.1.3.5Frozen Dough protein secondary structureFTIRDetermination

Said to take a certain amount of the sample and potassium bromide mass ratio1 100In agate mortar in grinding and mixed uniform suppress into thin slicesFTIRInstrument of samples do all-band (400~4 000 Length^{-1}) Scanning determination scanning number32Resolution4 Length^{-1}. After"Omnic 8.0Data Processing Soft automatic correction water vapor usePeakfit 4.12Software Interception1 600~1 700 Length^{-1}Band and the baseline correction Gaussian to Convolution second-order derivative fitting make residual greater0.96Identify protein secondary structure, peak and calculation the structure content.

1.3.6 Frozen Dough water loss rate of Determination

Will quick-frozen good Frozen Dough steamed bread every group out3A with wrap the plastic wrap placed-18℃ Refrigerator in Frozen Storage by frozen storage after freeze-thaw cycle treatment, press-calculation dough Water Loss Rate:

1.3.7 Frozen DoughNMRDetermination of relaxation time

UtilizationFIDThe resonance center frequency is adjusted by experiment.CpmgMeasurement of sample spin by Pulse Sequence-Spin Relaxation Time (T_2). Steamed dough (1.00±0.01)GPlaced in the test tube, placed in the center of the Permanent Magnetic FieldCpmgScanning experiment of pulse sequence.CpmgExperiment parameters: Sampling Points104 square meters 902 square meters, Repeat scan times8.Repeat time2 000 MS, Half echo time210 μs. UtilizationT_2Inversion Fitting SoftwareCpmgRelaxation Attenuation Curve inversion to get the relaxation map andT_2Value.

1.4 Data Processing

AdoptedSPSS 13.0Multiple comparisons of the mean (DuncanMethod), simple analysis and drawingOrigin 8.0Software.

2.1 Frozen Storage PeriodAFPsEffects on free sulfhydryl and two disulfide bond contents in Frozen Dough

Yutu1.Obviously, with the extension of frozen storage time, the content of free sulfhydryl groups in pre-fermented Frozen Dough increased, two the content of disulfide bonds decreased gradually. Therefore two The disulfide bond in

Frozen Dough broke with the extension of frozen storage time.Zhao LeiWait.[22]The research conclusion is consistent. Compared to the blank dough, the addedAFPsThe two content of disulfide bonds and sulfhydryl groups in Frozen Dough changed slightly, indicating thatAFPsTwo can reduce the break of disulfide bond in dough during freezing storage, and it can better protect the functional characteristics of gluten.

2.2 Freeze-thaw cycleAFPsEffects on free sulfhydryl and two disulfide bond contents in Frozen Dough

Yutu2.Obviously, with the increase of freeze-thaw cycles, the content of free sulfhydryl groups in Frozen Dough increased.5.After the second, addAFPsThe content of free sulfhydryl groups in Frozen Dough was lower than that in blank dough; conversely two the content of disulfide bonds in Frozen Dough was lower than that in freeze-thaw cycle.5.Two second, the content of disulfide bonds in the dough was higher than that in the blank dough.AFPsThe cleavage of two disulfide bonds in pre-fermented Frozen Dough was reduced by the addition.

Comparison chart1.And2.The results showed that the change trend of free sulfhydryl and two disulfide bonds in pre-fermented Frozen Dough During freeze-thaw cycles was the same as that in Frozen Dough, but the change range was higher than that in Frozen Dough, the results indicated that freeze-thaw treatment had more obvious damage to two disulfide bond in Frozen Dough. This may be because the repeated recrystallization of ice crystals in Frozen Dough caused by freeze-thaw cycles may aggravate the damage of dough gluten structure. ButAFPsThe modification of ice crystals and the inhibition of recrystallization can effectively inhibit the destruction of gluten structure during freezing and thawing.[23]And two reduce the fracture of disulfide bonds.

2.3 AFPsOn Frozen DoughSDS-PAGEInfluence

Yutu3.,4.We can know that frozen storage and freeze-thaw cycle are handled,100 kDaUp and down3.It shows that the molecular weight of this part of the subunit is the largest, the mobility is the slowest, and the color is shallow.HWM-GS;30~70 kDaThe content of the gel was higher, the migration rate was faster, and the molecular weight was smaller.LMW-GSAnd gliadin. Observe Frozen Storage and freeze-thaw cyclesSDS-PAGEIt was found that the number of bands and relative mobility of the Frozen Dough did not change significantly.AFPsHad no effect on Frozen Dough subunits.

2.4 Frozen Storage Conditions underAFPsThe Frozen Dough protein secondary structure of influence

FTIRAmideIWith each peak secondary structure identify reference von bud and[24]Methods. Frozen StorageAFPsThe Frozen Dough protein secondary structure of influence see Figure5.

Will2Of the Frozen DoughFTIRFigure usePeakfitSoftware the baseline correction Gaussian to Convolution after the protein secondary structure table peak of fitting protein secondary structure identify of the peak area change corresponding secondary structure of content also with the change. By figure5The After90 dFrozen Storage after,1 617 Length^{-1}The of molecularβ-Folding of content with frozen storage time of extended was rise Trend,1 669 Length^{-1}Theβ-Corner content was decreased trend and reverse parallelβ-Folding,α-Spiral, no rules Curly and1 683 Length^{-1}Theβ-Folding this4Kind of two level structure of content in frozen storage process in small fluctuation but change is not obvious. (Frozen storage will influence dough protein of molecularβ-Folding andβ-Corner of content and withβ-Corner of reduce molecularβ-Folding increase may isβ-Corner in frozen storage process in the transformation in orderβ-Folding and Zhao Lei[4]Research results consistent. From figure5Also can see addAFPs

Dough in protein secondary changes in the structure of blank dough low show thatAFPsOf to be able to join protection dough in protein molecular between of Hydrogen Bond role and protectionβ-Corner from ice crystals of damage.

2.5 Freeze-thaw cycle conditions underAFPsThe Frozen Dough protein secondary structure of influence

By figure6The freeze-thaw cycle process in2Kindβ-Folding content were was rise Trend,β-Corner content was decreased trend this and Frozen Dough in Frozen Storage Conditions under secondary structure change trend as but freeze-thaw cycle conditions

Under2Kind of two level structure of change amplitude significantly greater than frozen storage process,AFPsFrozen Doughβ-Folding reduce amplitude is blank group small. And frozen storage process different of is freeze-thaw cycle process inα-Spiral content was decreased trend. Freeze-thaw process due to temperature of continuous fluctuation and outside pressure of change lead to water migration and recrystallization big ice crystals quantity increased on protein in hydrogen bond play a destructive role, the protein of hydrophilic and hydrophobic residues exposure out protein molecular between and molecular in new of molecular cross-linked to change the protein of secondary structure. Li red and[25]Research have the same conclusion. AndAFPsOf to be able to join increase Gluten Protein of molecular in Hydrogen Bond role at the same time by promoting was damage of molecular between hydrogen bond of again formation Gluten Protein Secondary Structure keep stable state.

2.6 Frozen StorageAFPsThe Frozen Dough water loss rate of influence

By figure7/The pre-fermentation Frozen Dough with the frozen storage time of extended water loss rate significantly improve Frozen Storage15 dAfter blank group water loss rate significantly increase. This is because Frozen Dough in frozen storage when frozen storage room and dough between of steam pressure difference and Frozen Dough surface water constantly to ice crystals sublimation style reduce makes Frozen Dough internal water constantly out migration[26]Frozen Dough in water continuous reduce. Compared,AFPsFrozen Dough water loss rate increased trend significantly slow in blank group (in frozen storage process in,AFPsCan improve the dough of holding hydraulic effective to reduce Frozen Dough water of reduce (

AFPsIn certain degree on the can reduce dough can be frozen water of content to suppression water of loss[27].

2.7 Freeze-thaw cycle conditions underAFPsThe Frozen Dough water loss rate of influence

Yutu8.The results showed that the water loss rate of pre-fermented Frozen Dough increased significantly with the increase of freeze-thaw cycles. This is because in the process of freezing and thawing, acceleration

Water migration and recrystallization in the dough, which makes the ice crystals gradually[28-29]Large ice crystals further damaged the gluten network structure of the dough and reduced the water holding capacity of the dough. After repeated freeze-thaw treatment,AFPsThe water loss rate of Frozen Dough was lower than that of the blank group.AFPsThe addition of these compounds can slow down the loss of moisture in Frozen Dough During freeze-thaw cycles.AFPsIt can inhibit the recrystallization of ice crystals in the dough, reduce the production of large ice crystals, maintain the water holding capacity of the dough, and thereby reduce the rate of water loss in the dough.

2.8 Under freezing conditionsAFPsEffect on Water Distribution of Frozen Dough

AdoptedNMRThe water distribution in Frozen Dough was measuredCpmgSequence Determination Under Frozen Storage ConditionsAFPsSpin on frozen noodle Systems-Spin Relaxation TimeT_2.Change Impact. By table1.It is known that there are2.A suitable peak, of which1.A peakT_{21}.In1.~3 MSIt is characterized by water which is closely bound to protein, starch and other macromolecules.2.A peakT_{22}.In8.~13 MSThis part is generally considered as a weak-junction water layer that binds directly to strong bound water by hydrogen bonds and indirectly to macromolecules, comparedT_{21}.More liquid, semi-Combined Water[30]It shows that the water in the dough mainly exists in the combined water and the semi-combined water,A_{21}.,A_{22}.The higher the proton density is, the higher the water content is. But in the frozen dough30 dLater, the peaks in the map1.Hefeng2.Partial overlap, indicating that in this Transverse Relaxation time interval, the Frozen Dough^{1}HThe movement[31].

Note: different letters in the same column indicate significant differences,$P<0.05$The same below.-.No correlation peak was detected.

By table1.Frozen DoughT_2.With the extension of frozen storage time, the water fluidity increased, indicating that the freezing process accelerated the movement of moisture in Frozen Dough. In the tundra30 dAfter that, the Transverse Relaxation Time increased significantly ($P<0.05$), The combined water content decreased, and the semi-combined water content increased.60 dAfter that, the combined water and semi-Combined Water peaks almost coincide, and the content of semi-combined water increases significantly. This is due to the destruction of the dough gluten network structure by ice crystals generated during freezing storage, resulting in reduced water holding capacity and enhanced water fluidity of the dough.AFPsFrozen Dough has the same change trend as the blank group, addingAFPsThe relaxation timeT_2 Value has fallen, frozen60 d

After also can detection to bound water peak (AFPsCan effective suppression dough in water of migration improve Frozen Dough in frozen storage during the stability.

2.9 Freeze-thaw cycle conditions underAFPsThe Frozen Dough water distribution state of influence

By table2The freeze-thaw cycle3Times after two group Frozen DoughT_{21}AndT_{22}The peak location basic no change freeze-thaw cycle4Times after blank groupT_{22}Value significantly increase dough in water liquidity significantly improve addAFPsAfter,T_{22}Value basic constant show thatAFPsCan weakened freeze-thaw cycle on Frozen Dough water liquidity of influence.

In addition with the freeze-thaw cycle number of increase Frozen Dough in bound water content gradually reduce semi-bound water content significantly increase ($P<0.05$) Show that freeze-thaw cycle process in dough in bound water content decreased, semi-bound water content rise semi-bound water in surface is constantly growth and recrystallization, large ice crystals of increased and the dough of mechanical force lead to dough water holding capacity decreased water loss. And and blank group compared addAFPsAfter dough bound water and semi-bound water content change is blank group small this (AFPsCan reduce freeze-thaw cycle process in dough water of liquidity reduce water of migration.

By Frozen Storage and freeze-thaw cycle after frozen dough in free thiol content rise two sulfur key content decreased. With the frozen storage time of extended Secondary Structure in molecularβ-Folding content increase,β-Corner content reduce freeze-thaw cycle also will makeα-Spiral Structure content decreased addAFPsGroup of Frozen Dough protein characteristics change were less than without adding group so in Frozen Storage and freeze-thaw cycle process in,AFPsCan suppression two sulfur key of fracture and two level structure of change;SDS-PAGEMap Display Frozen Storage and freeze-thaw cycle for Frozen Dough protein of subunit no obvious influence and addAFPsThe Frozen Dough subunit no influence.

By Frozen Storage and freeze-thaw cycle after Frozen Dough of water loss rate significantly increased Relaxation TimeT_{21}AndT_{22}Value larger bound water content reduce semi-bound water increased show that frozen storage and freeze-thaw process enhanced the dough water of Flow

Of lead to dough water-holding capacity reduce System in Water molecules from combined with state to the semi-state change. AddAFPsAfter Frozen Dough water loss phenomenon has improve Frozen StorageT_{21}AndT_{22}Value decreased freeze-thaw cycle underT_{21}AndT_{22}Value basic constant bound water content rise reduce the freeze-thaw process in dough water of liquidity effective to improve the dough of holding water ability,AFPsCan improve Frozen Storage and freeze-thaw process in Frozen Dough steamed bread of quality.

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