

加水量对面筋蛋白水分分布及结构的影响

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摘要: 利用核磁共振仪(NMR)、扫描电子显微镜(SEM)、傅里叶变换红外(FITR)、差示扫描量热仪(DSC)和旋转流变仪(DHR)测定不同加水量下面筋蛋白的水分分布、微观结构、蛋白二级结构、热力学特性和流变学特性。结果表明,随加水量增大,面筋蛋白中结合水相对含量显著下降5.15个百分点,自由水相对含量显著升高8.21个百分点,弱结合水相对含量先增大后降低;面筋蛋白网络孔洞更加密集,孔径更加细小,但当加水量大于160%后,网络强度减弱;面筋蛋白二级结构表明,当加水量小于等于130%,随加水量增大,二级结构中 β -转角及无规则卷曲相对含量显著下降, α -螺旋、 β -折叠相对含量显著增大,当加水量大于130%,面筋蛋白二级结构变化趋势减缓,且无明显变化规律;面筋蛋白热变性峰值温度呈现先升高后降低的趋势,当加水量为150%时,取得最大值82.4℃,表明其热力学稳定性随水分增加而得到提升;面筋蛋白储能模量 G' 及损耗模量 G'' 均呈下降趋势,加水量大于130%时下降趋势减缓,且加水量150%时其弹性高于140%加水量。由此表明,150%是面筋蛋白的较优加水量。

关键词: 面筋蛋白; 加水量; 水分分布; 蛋白结构; 流变学特性

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Effect of Water Addition on Hydration and Structure of Gluten Protein

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Abstract: Effect of water addition on gluten protein was investigated by using nuclear magnetic resonance (NMR), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FITR), differential scanning calorimeter (DSC) and rotational rheometer (DHR). The results showed that the amount of water had a significant effect on the hydration, structure and functional properties of gluten protein. With the increase of water addition, the bound water content of gluten protein was decreased significantly from 22.42% to 17.27%, the free water content was increased significantly from 0.04% to 8.25%, and the weakly bound water content was increased first and then decreased, and the water was preferentially converted into bound water and weakly bound water. The gluten protein network had more dense pores and smaller pore size, but when the water was added more than 160%, the strength of the network was weakened. The secondary structure of gluten protein showed that when the water addition amount was not more than 130%, the β -turn angle and the random curl was decreased significantly with the increase of water addition, and the α -helix and β -folding were increased remarkably. When the water addition amount was more than 130%, the secondary structure change of gluten protein was slowed down, and there was no obvious changing rule. The heat denaturation temperature of the protein was firstly increased and then decreased. When the water addition amount reached 150%, the maximum value was 82.4℃, indicating that the thermodynamic stability was increased with the increase of water. The storage modulus G' and the loss modulus G'' of gluten protein all showed a downward trend. After the water addition was more than 130%, the downward trend was slowed down. When the water content was 150%, the elasticity was higher than 140%. The above results indicated that 150% may be the better water content of gluten protein, which also laid a foundation for further revealing the mechanism of water addition to wheat dough.

Key words: gluten protein; amount of water; water distribution; protein structure; rheological property

0 引言

面制品是我国北方的主要食物,食用历史悠久。近年来,通过对面团的外源添加及工艺改良等方式,在改善面团加工特性上进行了广泛研究^[1-2]。而在面制品形成过程中,水分的含量、分布及水合程度对最终制品的品质特性有着重要影响^[3-4]。文献[5]研究表明,含水率显著影响面团流变学特性,并使面包产品柔软性及粘弹性增强。文献[6]研究发现,水合程度对体外淀粉消化率及面制品品质起重要作用。小麦面筋蛋白是由具有弹性性能的谷蛋白和粘性性能的麦醇溶蛋白组成,作为面团的重要组成部分,其功能特性对面团品质有重要影响^[7]。研究表明,含水率对面筋蛋白与阿拉伯木聚糖复合体系的流变学特性有显著影响^[8]。同时,水合程度改变面筋蛋白二级结构,并对面团质构特性产生影响^[9]。文献[10]也发现,面筋蛋白二级结构因微晶纤维素减弱了面筋蛋白水合程度而改变。良好的水分分布可减弱冻藏期间因重结晶现象导致的面筋蛋白劣变,减少水分流失,从而保护面团加工性能^[11]。

面筋蛋白的水合程度对面团粘弹性及其制品的品质特性影响极大^[12-13],在目前面筋蛋白的研究中,加水量选取在100%~150%之间^[14-17],且不尽相同。本文采用核磁共振仪(NMR)、扫描电子显微镜(SEM)、傅里叶变换红外(FITR)、差示扫描量热仪(DSC)和动态流变仪(DHR)对面筋蛋白体系的水分分布、微观结构、蛋白二级结构、热力学及流变学特性进行测定,研究加水量对面筋蛋白的水合及结构的影响,分析水分在面筋蛋白中的作用机制,探讨面筋蛋白的较优加水量,以期改善小麦面团加工性能提供基础理论依据。

1 材料与方法

1.1 材料制备

将小麦面粉(一加一天然面粉有限公司,蛋白质质量分数13%)采用文献[14]的方法制得小麦面筋蛋白(蛋白质质量分数为87%、干基含水率为9%)。

1.2 方法

1.2.1 样品制备

将面筋蛋白与水均匀混合,制成1g的湿面筋蛋白块,加水量(水分与面筋蛋白的质量百分比)分别为110%、120%、130%、140%、150%、160%、170%、180%、190%,部分样品用于直接测定水分分布和流变学特性,部分样品置于-40℃低温冷柜速冻2h后,采用美国FTS公司Flexi-Dry型冷冻干燥

机冷冻干燥后粉碎、研磨,过100目筛置干燥器备用。

1.2.2 面筋蛋白中水分分布测定

采用上海纽迈电子科技有限公司Micro MR型核磁共振仪测定弛豫时间 T_2 。参数设置:共振频率为22 MHz,磁体温度为32℃,90°脉冲时间 P_{90} 为17 μ s,180°脉冲时间 P_{180} 为33 μ s,主频 S_w 为200 kHz,累加次数 N_s 为32,回波个数为5 000^[18]。

单位面筋蛋白中各水分相对质量计算公式为

$$m = AM$$

式中 m ——单位面筋蛋白中各水分相对质量,g

A ——单位面筋蛋白中各水分相对含量,%

M ——单位面筋蛋白中水分质量,g

1.2.3 面筋蛋白微观结构观察

冷冻干燥后样品表面喷金,采用美国FEI公司Quanta FEG-250型场发射扫描电镜进行500倍观察。

1.2.4 面筋蛋白二级结构测定

采用布鲁克科技有限公司TENSOR-II型傅里叶变换红外光谱仪测定面筋蛋白二级结构。参数设置:扫描范围400~4 000 cm^{-1} ,扫描32次,分辨率4 cm^{-1} ^[19]。对结果中酰胺I带(1 600~1 700 cm^{-1})位置去卷积、基线校正及二阶求导后进行曲线拟合,各特征峰与面筋蛋白二级结构对应关系参照文献[9]。

1.2.5 面筋蛋白热力学特性测定

蛋白热力学特性通过耐驰科学仪器商贸(上海)有限公司生产的DSC-214型差示扫描量热仪测定。称取5 mg冻干面筋蛋白粉,从30℃以10℃/min加热至150℃。起始温度(T_0)、热变性峰值温度(T_p)及焓值(ΔH)通过系统软件分析计算。

1.2.6 面筋蛋白流变学特性测定

动态流变学特性通过美国TA仪器有限公司生产的DHR-1型动态流变仪测定。参数设置:平板直径40 mm,夹具间隙2 mm,扫描频率0.01~100 Hz,应力1%,温度25℃^[20]。

1.2.7 数据分析

用SPSS 22.0对数据进行统计分析。

2 结果与分析

2.1 面筋蛋白水分分布

由图1可看出,面筋蛋白中水分由结合水(弛豫时间 T_{21})、弱结合水(弛豫时间 T_{22})、自由水(弛豫时间 T_{23})3部分组成, T_{21} 、 T_{22} 、 T_{23} 峰值面积占总面积比例 A_{21} 、 A_{22} 、 A_{23} 分别反映不同形式的水相对含量^[21]。由表1可知,随加水量增大,面筋蛋白中结

合水相对含量显著下降 5.15 个百分点,自由水相对含量显著升高 8.21 个百分点,弱结合水相对含量呈现先增大后降低的趋势,这与文献[4]研究结果相一致。图 2~4(图中不同字母表示差异显著)为单位面筋蛋白中各水分相对质量,可知当加水量小于等于 140% 时,随加水量增加,面筋蛋白中结合水相对质量显著降低,弱结合水和自由水相对质量均显著增大,而当加水量大于 140% 时,随加水量增加,结合水相对质量呈先增大后平缓的趋势,弱结合水相对质量趋于平缓,自由水相对质量仍显著升高。实验表明面筋蛋白中水分优先转化为结合水和弱结合水,随加水量的增大,水分流动性增强并向弱结合水聚集,导致结合水含量下降,当加水量大于等于 150% 后,结合水和弱结合水基本饱和,出现平缓趋势,多余水分转化为自由水。这可能是因为在低水合状态时,蛋白质之间因谷氨酰胺残基的链间氢键相互作用,随着水合程度增加,链之间形成水合氢键结构,而高水合作用导致水和谷氨酰胺形成氢键,又破坏了链间相互作用,从而引发不同水分的转化,同时这也与蛋白二级结构中 β -转角相关联^[22]。这可能说明当加水量大于等于 150% 时,面筋蛋白中水分趋于稳定状态。

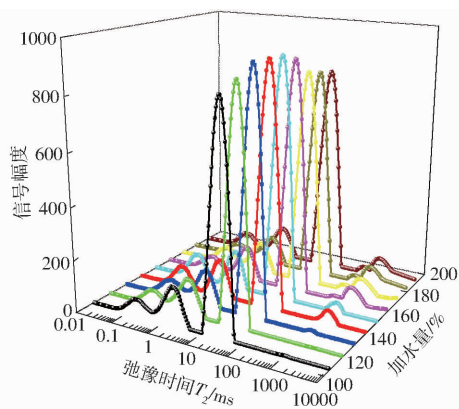


图 1 加水量对面筋蛋白弛豫时间 T_2 的影响

Fig.1 Effect of water addition on gluten protein T_2

表 1 加水量对面筋蛋白各水分相对含量的影响

Tab.1 Effect of water addition on relative water contents of gluten protein %

加水量	A_{21}	A_{22}	A_{23}
110	(22.42 ± 0.15) ^a	(77.55 ± 0.16) ^{bc}	(0.04 ± 0.02) ^a
120	(21.04 ± 0.26) ^b	(78.74 ± 0.29) ^b	(0.22 ± 0.05) ^a
130	(19.45 ± 0.18) ^c	(79.77 ± 0.29) ^a	(0.78 ± 0.12) ^{ab}
140	(18.36 ± 0.16) ^{cd}	(80.38 ± 0.19) ^a	(1.26 ± 0.30) ^b
150	(18.26 ± 0.44) ^{cd}	(79.31 ± 0.46) ^{ab}	(2.44 ± 0.14) ^c
160	(18.34 ± 0.68) ^{cd}	(77.25 ± 0.44) ^c	(4.41 ± 0.30) ^d
170	(18.17 ± 0.22) ^{cd}	(75.76 ± 0.24) ^d	(6.07 ± 0.31) ^e
180	(17.67 ± 0.50) ^d	(73.89 ± 0.31) ^e	(8.43 ± 0.19) ^f
190	(17.27 ± 0.26) ^d	(74.48 ± 0.49) ^e	(8.25 ± 0.27) ^f

注:同列数据不同字母表示有显著性差异($P \leq 0.05$)。下同。

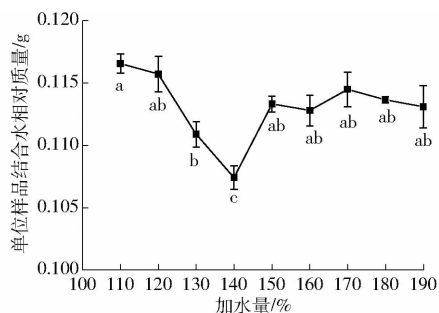


图 2 单位面筋蛋白中结合水相对质量

Fig.2 Relative mass of bound water in unit gluten protein

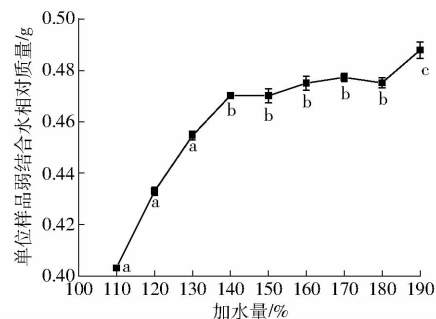


图 3 单位面筋蛋白中弱结合水相对质量

Fig.3 Relative mass of weakly bound water in unit gluten protein

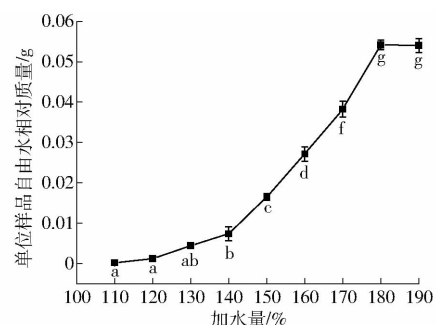


图 4 单位面筋蛋白中自由水相对质量

Fig.4 Relative mass of free water in unit gluten protein

2.2 面筋蛋白微观结构

图 5 直观显示了不同加水量下面筋蛋白三维网络结构,其中谷蛋白聚集体形成连续纤维状贯穿整个面筋网络,构成网状结构的“骨架”,醇溶蛋白以球状蛋白形式填充在谷蛋白网络结构中,蛋白网络孔洞是面筋蛋白速冻后冰晶体经脱水而形成^[18]。由图 5a、5b 可知,当面筋蛋白中加水量较低时,面筋网络中孔洞较少,孔壁较厚,且孔径可达 300 μm 左右。130% 的加水量使面筋网络孔径迅速减小至 80~100 μm ,随加水量再次加大,面筋网络更均匀,孔洞更密集、细小,且孔径多在 20 μm 以下。由图 5f~5i 可看出,当加水量大于 160% 时,面筋网络虽显示出更致密的孔洞,但面筋网络结构出现松散,网络强度减弱。这是因为低加水量下面筋网络无法充分形成,而较厚的孔壁会使气体保持能力下降,并影响加工特性^[23],随水分增多,水通过链间氢键,疏

水相互作用和巯基二硫化物交换反应促进面筋网络的形成^[24],而过多的水分可能稀释了为面筋蛋白提

供粘性并赋予其强度和弹性的醇溶蛋白^[25],并导致其网络强度变差。

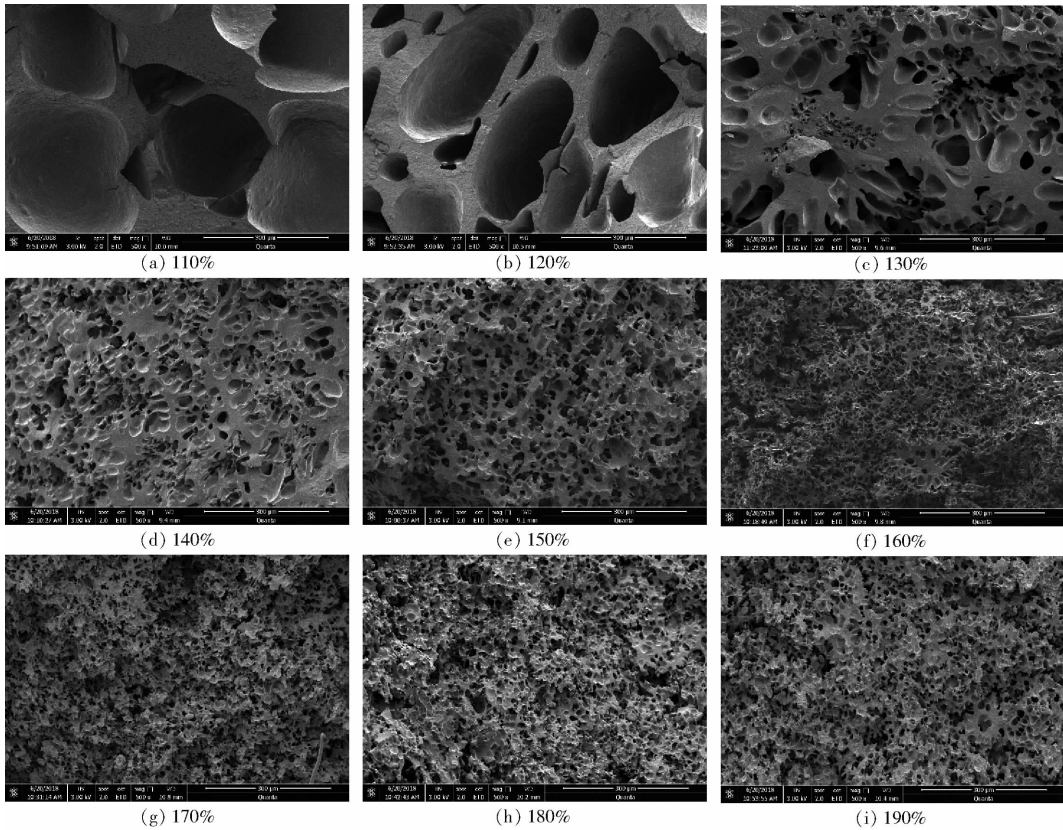


图5 不同加水量下面筋蛋白的微观结构($\times 500$)

Fig.5 Microstructures of gluten protein under different water additions ($\times 500$)

2.3 面筋蛋白二级结构

如表2所示,在加水量为110%时,面筋蛋白二级结构中 β -折叠、 α -螺旋、 β -转角及无规则卷曲相对含量分别为49.56%、11.81%、12.72%、25.92%。随水分的增加, β -转角及无规则卷曲相对含量显著下降, α -螺旋、 β -折叠相对含量显著增大。 α -螺旋、 β -折叠为稳定性较好的有序蛋白二级结构, β -转角和无规则卷曲为无序结构^[26],这表明水分增大使得面筋蛋白形成更稳

定的体系。这是因为低水分条件下面筋蛋白是通过紧密堆积的氢键形成无序结构,随水合程度增大,水和谷氨酰胺侧链之间的氢键竞争及链间相互作用形成有序列的区域,使其稳定性提升^[27]。实验发现当加水量超过130%,二级结构变化趋势减弱,且无明显规律,但在150%时 α -螺旋、 β -折叠相对含量总和最大。结果也与面筋蛋白水分分布情况及微观结构相对应,表明在加水量150%时,面筋蛋白达到较稳定状态。

表2 加水量对面筋蛋白二级结构相对含量的影响

Tab.2 Effect of water addition on secondary structure of gluten protein

加水量	α -螺旋	β -折叠	β -转角	无规则卷曲
110	(11.81 \pm 0.10) ^a	(49.56 \pm 0.06) ^a	(12.72 \pm 0.11) ^a	(25.91 \pm 0.15) ^a
120	(12.77 \pm 0.16) ^b	(49.39 \pm 0.80) ^a	(12.60 \pm 0.08) ^a	(25.24 \pm 0.72) ^a
130	(24.31 \pm 0.04) ^{cd}	(50.94 \pm 0.21) ^b	(11.95 \pm 0.14) ^b	(12.80 \pm 0.12) ^b
140	(24.23 \pm 0.07) ^{cd}	(51.57 \pm 0.16) ^b	(11.68 \pm 0.12) ^b	(12.52 \pm 0.21) ^b
150	(24.35 \pm 0.08) ^{cd}	(51.95 \pm 0.23) ^b	(11.39 \pm 0.14) ^b	(12.32 \pm 0.09) ^b
160	(23.90 \pm 0.17) ^c	(52.37 \pm 0.43) ^b	(11.17 \pm 0.02) ^c	(12.56 \pm 0.28) ^b
170	(24.42 \pm 0.13) ^d	(51.43 \pm 0.14) ^b	(11.65 \pm 0.06) ^b	(12.51 \pm 0.07) ^b
180	(24.27 \pm 0.03) ^{cd}	(51.61 \pm 0.05) ^b	(11.77 \pm 0.01) ^b	(12.34 \pm 0.04) ^b
190	(24.20 \pm 0.10) ^{cd}	(51.25 \pm 0.02) ^b	(11.76 \pm 0.02) ^b	(12.80 \pm 0.08) ^b

2.4 面筋蛋白热力学特性

由表3可知,随加水量增大,面筋蛋白热变性起始温度由41℃左右显著增大至约52℃,表明随加水量增大面筋蛋白结构逐渐趋于稳定,蛋白变性难度增大。热变性焓值显著下降表明面筋蛋白中疏水性基团的减少,这有利于面筋蛋白网络结构的展开^[28]。热变性峰值温度呈现先升高后降低的趋势,在加水量150%时热变性峰值温度取得最大值82.4℃,这可能是由于随水分增多,热变性温度随蛋白聚集而增大^[28]。这表明加水量的增大有效提升了面筋蛋白的热力学稳定性,而过多的水分可能会导致面筋蛋白网络松散,并降低热变性温度。

表3 加水量对面筋蛋白热力学特性的影响

Tab.3 Effect of water addition on thermodynamic properties of gluten protein

加水量/%	$T_0/^\circ\text{C}$	$T_p/^\circ\text{C}$	$\Delta H/(\text{J}\cdot\text{g}^{-1})$
110	(40.95 ± 0.57) ^a	(72.80 ± 0.79) ^a	(6.42 ± 0.52) ^a
120	(41.23 ± 0.59) ^a	(74.05 ± 0.48) ^a	(5.81 ± 0.45) ^{ab}
130	(41.00 ± 0.10) ^a	(76.10 ± 0.66) ^b	(5.62 ± 0.18) ^{ab}
140	(41.23 ± 0.23) ^a	(78.83 ± 0.70) ^c	(5.18 ± 0.31) ^b
150	(44.15 ± 0.76) ^b	(82.40 ± 1.73) ^d	(4.11 ± 0.32) ^c
160	(45.30 ± 0.56) ^b	(79.10 ± 1.70) ^c	(4.29 ± 0.34) ^c
170	(53.60 ± 1.11) ^c	(78.60 ± 1.10) ^c	(3.29 ± 0.89) ^c
180	(53.13 ± 1.33) ^c	(78.10 ± 1.00) ^c	(3.95 ± 0.33) ^c
190	(51.83 ± 1.46) ^c	(79.23 ± 0.55) ^c	(3.09 ± 0.74) ^c

2.5 面筋蛋白流变学特性

如图6所示,面筋蛋白储能模量 G' 和损耗模量 G'' 均随频率增加而增大,且 G' 始终大于 G'' ,具有类似固体性质^[29]。随加水量增大,面筋蛋白弹性及粘性均呈现下降趋势。这是因为随水分增多蛋白质面筋逐渐形成,面筋蛋白延伸性提升,致使其粘弹性发生改变^[30]。文献[9]也发现面团弹性性能随加水量增大而降低。当加水量大于130%时,弹性与粘性下降趋势明显减缓,这可能与面筋蛋白二级结构的改变相关^[31]。实验发现面筋蛋白加水量为150%时,其弹性性能高于加水量为140%时,且粘性性能与之接近,这可能因为加水量150%时面筋网络充分形成,且网络强度较大。这表明在面筋网络充分形成的前提下,150%是面筋蛋白加工生产的较优加水量。

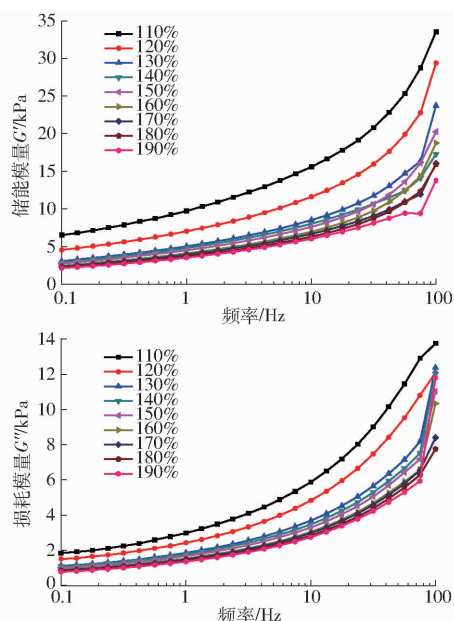


图6 加水量对面筋蛋白动态流变学特性的影响
Fig.6 Effect of water addition on dynamic rheological properties of gluten protein

3 结论

(1)在面筋蛋白水合过程中,水分优先转化为结合水与弱结合水,随加水量增大,结合水含量显著下降,自由水含量显著上升,弱结合水含量先增大后降低,且加水量大于等于150%时,面筋蛋白水分状态趋于稳定。

(2)随加水量增大,蛋白网络孔洞更密集,孔径更小。二级结构中 α -螺旋、 β -折叠相对含量显著增大,加水量150%时总和为最大,显示出更稳定状态。这使得蛋白热变性温度提升,热稳定性得到改善,但水分过多会使面筋网络强度减弱,稳定性降低。

(3)随加水量增大面筋蛋白弹性及粘性均呈下降趋势,加水量大于130%时下降趋势减缓,且加水量150%时其弹性高于140%加水量。

(4)面筋蛋白加水量在150%时,其水分分布状态趋于稳定,良好的水分分布促使面筋蛋白网络充分形成,并对粘弹性产生有利影响,其二级结构及热力学特性也均表现出较优、较稳定状态,这表明此加水量是面筋蛋白的较优加水量。

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