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Physicochemical Assessment of Quality Characteristics of Extruded Barley Under Varied Storage Conditions

II. Non-enzymatic Browning

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상이한 조건하에서 저장한 압출보리의 품질특성에 관한 이화학적 평가

제 2 보 : 비효소적 갈색화

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Abstract

Change of color, browning index, soluble protein, reducing sugar content, and available lysine were monitored for raw and extruded barely powders during four months of ambient and accelerated storage temperatures with A_w of 0.31 and 0.71, respectively. Loss of whiteness and soluble browning pigments increased with increased A_w and temperature. The raw sample had an increased rate of browning intensity than the extruded samples. Among the extruded samples, the added sucrose sample had the lowest rate of browning during storage. Loss of reducing sugar content in all samples increased with increased A_w and temperature. The loss of soluble protein and of the available lysine also increased with increased A_w and temperature. The loss of reducing sugar and of the available lysine was at least partly due to the Maillard browning reaction. These results have important implications in teh processing and storage of raw and extruded barleys.

Introduction

In the previous paper[1], we reported the stability of raw and extruded barleys with respect to lipid oxidation under varied conditions of temperature and water activity. Non-enzymatic browning reactions, during food storage, are dependent on such factors as moisture content, storage temperature, and storage atmosphere. Lea and Hannan[2] studied the effect of water activity on disappearance of amino nitrogen in casein-glucose mixture. They found a maximum reaction at 65~70% equilibrium relative humidity. In dry milk, Loncin *et al*[3] found a maximum loss of lysine and in the rate of browning within the same range of water activities. Dried meat showed maximum browning at an equilibrium

relative humidity of 57%[4] and pea soup mix at 70%[5]. Another study to non-enzymatic browning derives its carbonyl reactions from autoxidizing lipids. Oxidized lipids emulsified in aqueous dispersions of proteins gave brown copolymers[6] and complexes[7]. In the report of Fragne and Adrian[8], lysine, methionine, and the N-terminal amino acids of proteins were indicated to react most strongly with reducing sugars. The loss of lysine that have bewen demonstrated in model systems of proteins and sugars were obtained under rather drastic conditions that do not occur during normal processing of high protein foods. However, looses of these amino acids in breakfast cereals may be significant be cause they are not abundant at the outset.

At present, very little of the literature deals with the effect of temperature and water activity on the non-enzymatic browning of breakfast cereals during storage. In this study, changes of color, browning index, soluble protein, reducing sugar content, and available lysine in the raw and extruded barleys were measured at four month interval during storage. The purpose of this investigation was to study the effect of storage temperatures and water activities on related non-enzymatic browning.

Materials and Methods

Preparation of extruded barley and storage conditions

The preparation method of extruded barley and storage conditions were treated as described in previous paper[1].

Non-enzymatic browning pigments

The formation of water soluble brown pigments was measured according to the method of Choi *et al.*[9] as modified by Karel and Labuza[10]. One gram samples were dispersed in 30 *ml* of water, and 2.5 *ml* of a 10% freshly prepared suspension of trypsin were added. After an hour of incubation at 45°C, 2 *ml* of 50% trichloroacetic acid was added. After mixing and centrifugation (18,000 *rpm* for 20 *min*), the absorbance at 400 *nm* was measured on the clear solution, with enzyme blank set at 100% transmittance. The results were reported as (absorbance per gram solid) × 100.

Color analysis

The color changes of samples were also monitored by using the Hunter Colorimeter Model D 25-2 (Hunter Associates Laboratories Inc., VA). Enough amounts of samples were added to the colorimeter cup to cover the bottom of the cup such that there were no transparent areas. After standardizing the equipment with a white plate give L, a, b values of the standard supplied with equipment, "L" values which measure the degree of whiteness of the samples were determined.

Soluble protein determinations

Soluble protein was measured by dispersing one gram of samples into 30 *ml* of 8*M* urea-1N NaOH (1:1, v/v) which was contained in a 50 *ml* polypropylene centrifuge tube. After 40 *ml.* the dispersions were centrifuged (15,000 *rpm* for 20 *min*) and 2 *ml* aliquots of the supernatant were analyzed for the protein content by the Biuret procedure[11].

Total reducing sugar determinations

Analyses of reducing sugar content were performed according to AOAC method[12].

Available lysine determinations

The available lysine was determined by the method of Peterson and Warthesen[13]. Approximately one gram samples were accurately weighed and placed in boiling flask. Ten *ml* of 1-fluoro-2,4-dinitrobenzene (Eastman Kodak Co.) were added. The sample was shaken for 4 hours at room temperature by a Burrell wrist action shaker (Burrell Corp.) and the ethanol was then evaporated until weight loss of 12.5 g was obtained. Thirty *ml* of 8.1*N* HCl was added and the sample was refluxed for 16 hours. After the acid hydrolysis, the sample was filtered while still hot and brought to a volume of 250*ml* with water. Approximately one *ml* was then filtered through a 0.45 *um* membrane filter (Gelman Metricel). Separation and quantitation of DNP-lysine was then accomplished by HPLC.

The liquid chromatograph consisted of a Waters Associated Model 6000 *A* pump, U6K injector and 440 absorbance detector fitted for determination of wavelengths of 436 or 254 *nm*. The detector output was recorded on a Hewlett Packard 3380 *A*. The wavelength used to detect DNP-lysine was 436 *nm*. The separation was accomplished on a *M* Bondapak C_{18} column (3.9*mm* id × 30 *cm*, Waters Associates) with a mobile phase of 20% nanograde acetonitrile (Mallinckrodt) and 80% 0.01M acetate buffer, pH 4.0. With a flow rate of 1.0 *ml* per minute, DNP-lysine eluted in 20-21 minutes. The usual volume injected was 80-100 *μl*. For peak identification and quantitation, DNP-lysine HCl (Sigma

Chemical Co.) was used as an external standard. A 2,4-dinitrophenol standard was also used to identify it together with the DNP-lysine.

Results and Discussion

Color changes and browning

The changes of "L" values and browning indices in the stored raw and extruded barley powders is presented in Table 1 and 2, respectively. The tables shows loss of whiteness and increase of soluble browning pigments with increasing A_w and with increasing temperature and storage time.

Loss of whiteness was obviously due to browning, the which may have been caused by the Maillard type reactions between the epsilonamino group of lysine and other such reactive amino groups in various kinds of compounds such as the phospholipids and the carbonyl groups of such compounds an reducing sugars and lipid oxidation products such as malonaldehyde, aldehydes and ketones. The literature cites many such instances[14-17].

Table 1 shows rate of browning intensity (decrease in "L" values per one month interval) of the raw samples to be greater than that for the extruded samples. This may be due to the activities of enzyme such as polyphenolase, amylase hydrolytic enzymes and lipoxygenase which may have been still active in the raw sample. As shown in the Table 1, the extruded sample containing added sucrose had lower browning intensity rate than the other extruded barley samples. However, the decrease of "L" values in Table 1 and increase of soluble browning pigments in Table 2 were not consistent with each other. The cause of this inconsistency requires further research. However, in this study, the stored extruded barley samples had less intense color changes, i.e. browning index. The change of color should not be a problem in the extruded barley powders provided it does effect nutritional value.

Changes in reducing sugar

The percentage changes of the reducing sugar in the stored raw and extruded barley poweders are shown in Fig. 1. Increasing the A_w from 0.31 to 0.75 led to decrease in the percentage amounts of reducing sugar in the all samples. The effect of increasing the temperature from 25 °C to 40 °C similarly caused decrease in the amount of reducing sugar. An explanation for this loss would appear to be that higher A_w and temperature promoted greater browning reaction involving the carbonyl groups of the reducing sugars and reactive amino groups

Table 1. Effects of water activities, temperatures and storage time on the browning intensity ("L" value) of raw and extruded barley powders

Storage time *(months)*	0.31 A_w							
	Raw		Run 1		Run 2		Run 3	
	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C	25 °C	40 °C
0	85.04	85.04	76.58	76.58	74.08	74.08	73.42	73.42
1	84.91	84.85	76.32	76.46	74.09	73.87	73.27	73.28
2	84.84	84.51	76.02	75.82	73.91	73.55	73.04	72.85
3	83.82	83.69	75.94	75.42	73.43	72.81	72.89	72.43
4	83.42	83.02	75.54	75.16	72.89	72.22	72.68	72.17
Rates[a]	0.405	0.505	0.255	0.355	0.198	0.465	0.185	0.313
	0.75 A_w							
1	84.76	84.63	76.25	76.31	74.01	73.45	73.18	72.84
2	84.24	84.09	75.63	75.43	73.43	72.69	72.96	72.02
3	83.68	83.11	75.21	74.81	72.86	72.04	72.63	71.45
4	83.07	82.43	74.94	74.28	72.13	71.57	71.94	70.62
Rates[a]	0.493	0.653	0.410	0.575	0.488	0.628	0.370	0.700

[a] The rates are decrease in "L" values per one month interval.

Table 2. Effects of water activities, temperatures and storage time on the browning indices of raw and extruded barley powders[a]

Storage time (months)	0.31 A_w							
	Raw		Run 1		Run 2		Run 3	
	25℃	40℃	25℃	40℃	25℃	40℃	25℃	40℃
0	4.51	4.51	22.62	22.62	18.49	18.49	19.93	19.93
1	4.96	5.14	22.99	23.59	18.54	18.93	20.51	21.35
2	5.61	5.93	23.65	24.17	19.68	19.95	21.38	21.94
3	6.34	6.85	24.05	25.21	20.41	21.93	21.94	22.65
4	6.82	7.45	25.34	26.69	21.08	22.36	22.37	23.48
Rates[b]	0.578	0.735	0.678	1.018	0.648	0.968	0.610	0.880
	0.75 A_w							
1	4.90	5.69	23.11	23.72	18.62	19.34	20.52	21.45
2	5.91	6.81	23.86	25.44	20.25	20.67	21.67	22.53
3	6.83	7.43	24.85	26.75	21.34	22.25	22.32	23.93
4	7.69	9.27	25.93	28.87	22.69	24.89	22.98	24.25
Rates[b]	0.698	0.895	0.823	1.563	1.050	1.600	0.763	1.080

[a] The index was expressed as 100 × absorbance at 400 *nm* per gram solid.

[b] The rates are increase per one month interval.

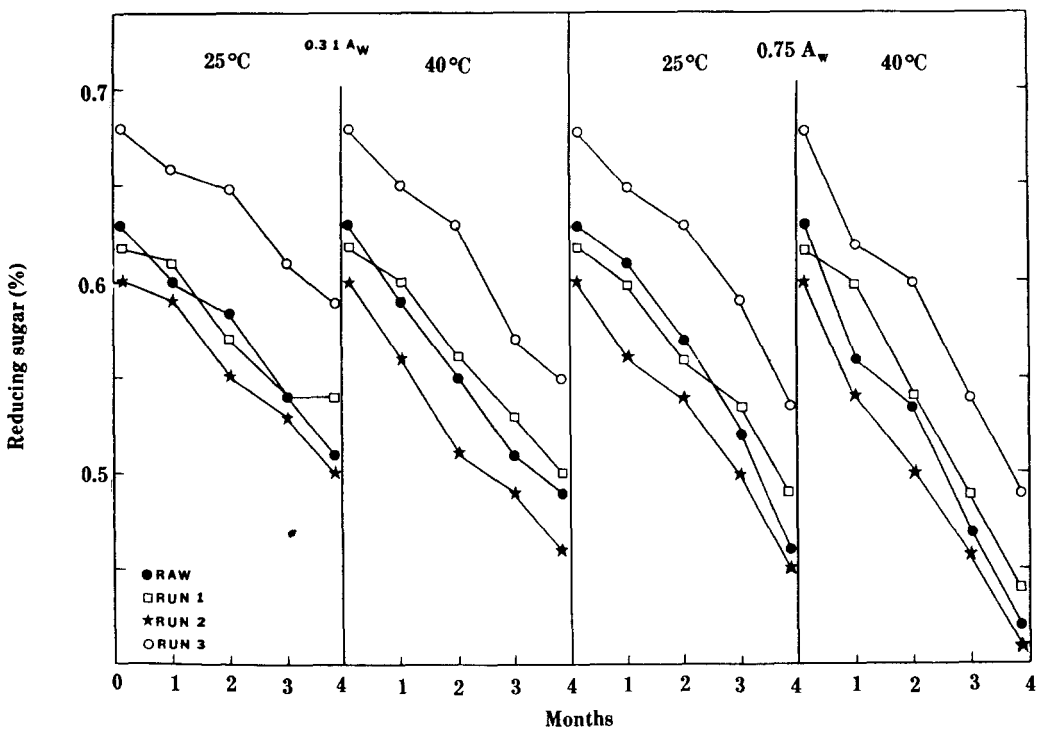

Fig. 1. Effects of water activities, temperatures and storage time on the reducing sugar content of raw and extruded barley powders

in the samples. The speculation here is that Maillard browning may account for the observed trend with is consistent with the reports of many workers in the field[18,19].

The amount of reducing sugar at 0.31 A_w was higher than that at 0.75 in this study. This is because the browning intensity is lower at the lower A_w because the degree of water binding at the lower A_w is greater and thus reactions depending on a solution such as Maillard browning would be slower at the lower $A_w$than higher A_w. Non-enzymatic browning may have been accelerated at the higher A_w's because the reactants were water soluble. Love and Dugan[19], and Ukhum[20] reported a similar loss of reducing sugars as the A_w was increased from 0.33 to 0.71.

Increasing storage time led to losses of reducing sugar content in the all samples. The calculated rates of loss (decrease in percentage reducing sugar per one month interval) of reducing sugars are given in Table 3. The rate of loss was slightly higher in the raw sample than in the extruded samples. In the raw sample, browning phenomenon may have been coupled with other phenomena which would account for the higher rate of reducing sugar loss. With the enzyme system intact, mitochondrial linked respiration starting with the glycolytic pathway of sugar metabolism is possible. It might be expected that since the hydrolytic enzymes responsible for the release of reducing sugars from their complex carbohydrate polymers would be expected to be functional in the raw powder, their rate of reducing sugars loss should be lower.

Table 3. Calculated rates of loss of reducing sugars content in stored raw and extruded barley powders[a]

Sample	0.31 A_w		0.75 A_w	
	25 ℃	40 ℃	25 ℃	40 ℃
Raw	0.028	0.038	0.043	0.053
Run 1	0.020	0.030	0.035	0.045
Run 2	0.025	0.035	0.038	0.050
Run 3	0.025	0.033	0.038	0.048

[a]The rates are decrease in percentage reducing sugar per one month interval.

Changes in soluble protein

The changes in the water soluble protein content of the stored raw and extruded barley powders are shown

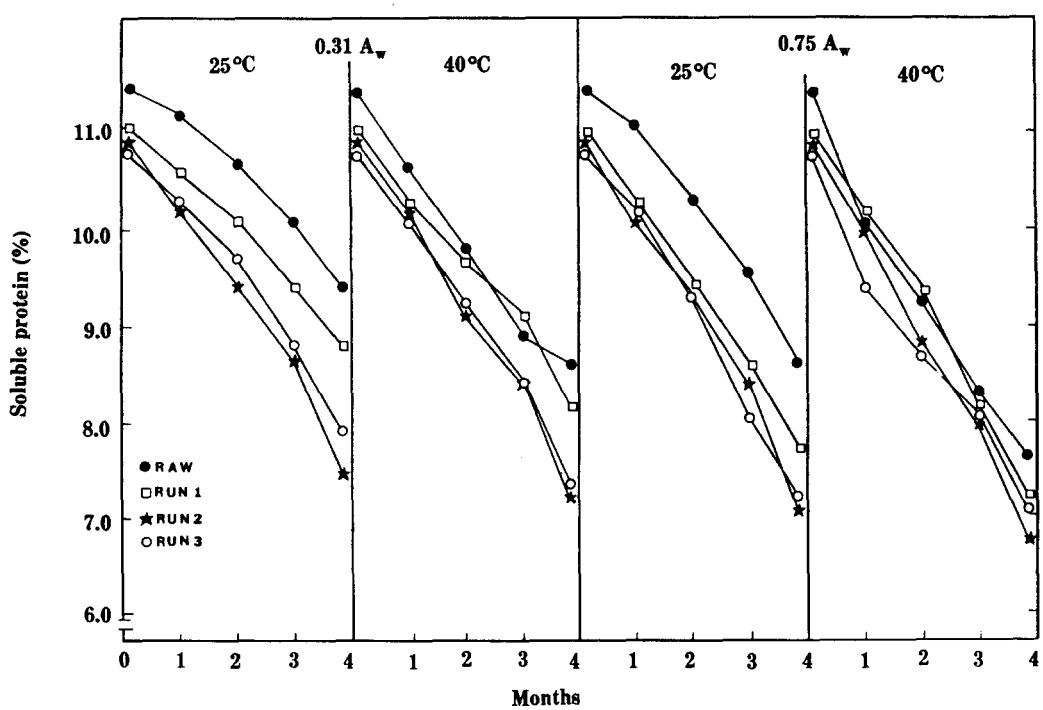

Fig. 2. Effects of water activities, temperatures and storage time on the soluble protein content of raw and extruded barley powders

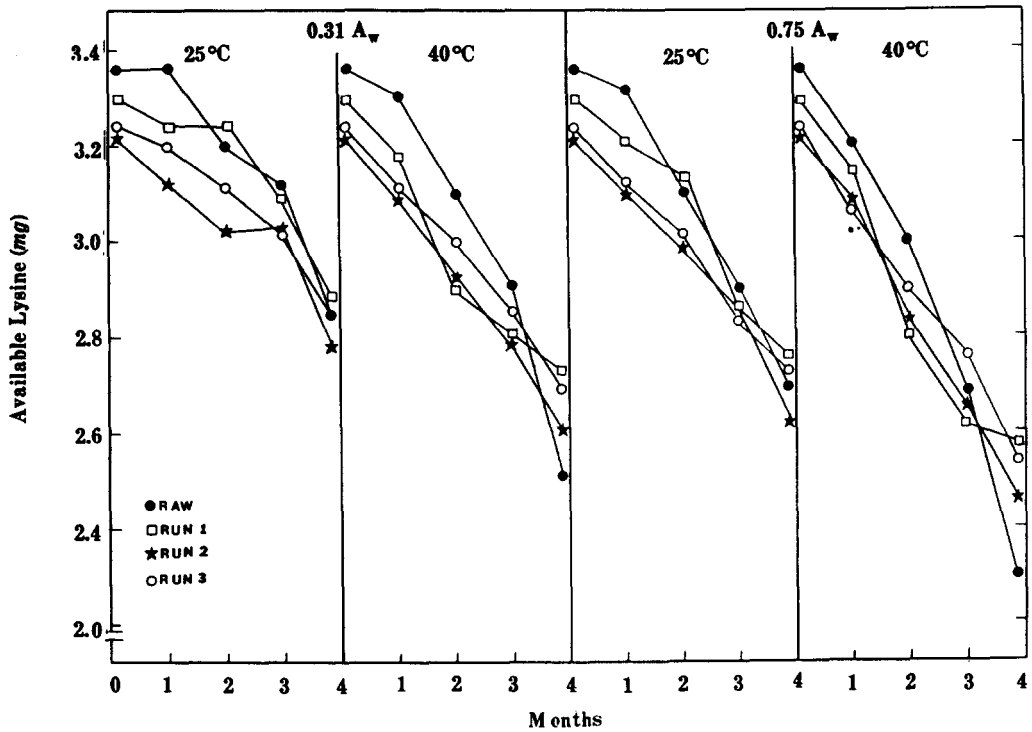

Fig. 3. Effects of water activities, temperatures and storage time on the available lysine content of raw and extruded barley powders.

in Fig. 2. The water soluble protein content of the raw and extruded barley powders tended to decrease as the A_w was increased. The same tendency was exhibited when the temperature was increased. The explanation for the above is most likely to be because of increased Maillard browning as both the A_w and temperature were increased.

The trend reported here is supported by the findings of other workers in the field. Working with unsaturated fats which had been oxidized and with proteins, Tappel[21] was able to state that these unsaturated fatty acids reacted with protein to yield stable complexs with decreased solubility. Schwenke[14] noted that some of the protein characteristics affected by Maillard browning reactions were the iso-electric point, electrophoretic behavior, solubility and precipitation characteristics.

McInroy *et al.*[22] reported that increased browning led to decreased solubility and digestibility of protein. In this study, the differences between the water soluble protein contents of the all extruded barley powders at the initial and at the end of storage appear to be marked. This is important in view of the associated implications of lowered protein solubility and digestibility. Since the results of this study shows that a lower A_w and temperature were effective in maintaining the soluble protein content of the extruded barley powders, it is recommended that low temperature and $A_w$storage be used to store this rather new product.

Table 4 shows the calculated rate of loss (decrease in percentage soluble protein per one month interval) of water soluble proteins over the four months storage period. The results agree with the trend previously discussed. The slightly lower value for the raw sample

Table 4. Calculated rates of loss of soluble protein content (%) in stored raw and extruded barely powders[a]

Sample	0.31 A_w		0.75 A_w	
	25°C	40°C	25°C	40°C
Raw	0.505	0.708	0.710	0.958
Run 1	0.545	0.715	0.830	0.980
Run 2	0.868	0.928	0.973	1.045
Run 3	0.738	0.885	0.920	0.965

[a] The rates are decrease in percentage soluble protein per one month interval.

than the extruded samples is difficult to explain.

Changes in available lysine

The changes in the available lysine content of the stored raw and extruded barley powders are shown in Fig. 3. The available lysine content of the raw and extruded barley powders tended to decrease as the A_w and temperature were increased, while the browning increased. The ability of the epsilon-amino group of lysine to enter into Maillard reactions with the carbonyl groups of such compounds as reducing sugars and oxidation products such as malonaldehyde is well documented. According to Carpenter and Booth[23], the lysine portion of a protein is able to enter into such reactions because it is the only essential amino acid that still has a free amino group in its condensed form as it exists in the protein molecule. The effects of temperatures and water activities on the loss of available lysine content in dry foods during storage has been investigated by many authors[20, 24-26]. Since available lysine content during storage indicates a decrease in protein quality. This was especially true when samples were stored at a higher temperature and/or higher water activities.

Table 5. Calculated rates of loss of available lysine content (*mg/g* powder) in stored raw and extruded barley powders[a]

Sample	0.31 A_w		0.75 A_w	
	25℃	40℃	25℃	40℃
Raw	0.130	0.205	0.155	0.268
Run 1	0.103	0.145	0.135	0.183
Run 2	0.113	0.150	0.148	0.188
Run 3	0.100	0.138	0.133	0.185

[a] The rates are decrease in available lysine content per one month interval.

The calculated rates of loss, decrease in available lysine (*mg/g* powder) one month interval, of available lysine content are given in Table 5. The rate of loss was slightly higher in the raw sample than in the case of browning and with other measures of Maillard browning such as the rate of loss of reducing sugar.

요 약

3가지 다른 조건하에서 제조된 압출보리와 생보리를 0.31 및 0.71의 수분활성과 25℃ 및 40℃의 온도에서 각각 4개월 저장하면서 색도, 갈색화지수, 가용성 단백질, 환원당 및 유효성 리진의 변화에 대한 수분활성과 온도의 영향에 대하여 연구하였다. 저장기간의 경과와 함께 수분활성과 온도가 증가함에 따라 모든 시료에서 백색도의 손실이 증가하였다.

생보리는 압출보리보다 저장중 갈색화 지수의 증가가 심하였고, 설탕을 첨가하여 제조한 압출보리는 다른 압출보리보다 저장중 갈색화가 적었다.

수분활성과 온도가 증가함에 따라 모든 시료중의 환원당, 가용성 단백질 및 유효성 리진의 함량이 감소하였으며, 이와같은 결과는 저장중 Maillard반응에 의한 것임을 알았다.

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