International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:4, No:7, 2010

Optimization of Enzymatic Activities in Malting of Oat

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***Abstract*—**Malting is usually carried out on intact barley seed, while hull is still attached to it. In this study, oat grain with and without hull was subjected to controlled germination to optimize its enzymes activity, in such a way that lipase has the lowest and α-amylase and proteinase the highest activities. Since pH has a great impact on the activity of the enzymes, the pH of germination media was set up to 3 to 8. In dehulled oats, lipase and α-amylase had the lowest and highest activities in pHs 3 and 6, respectively whereas the highest proteinase activity was evidenced at pH 7 and 4 in the oats with and without hull respectively. While measurements indicated that the effect of hull on the enzyme activities particularly in lipase and amylase at each level of the pH are significantly different, the best results were obtained in those samples in which their hull had been removed. However, since the similar lipase activity in germinated dehulled oat were recorded at the pHs 4 and 5, therefore it was concluded that pH 5 in dehulled oat seed may provide the optimum enzyme activity for all the enzymes.

***Keywords*—**Enzyme activity, malting, oat, optimization.

I. INTRODUCTION

OAT (*Avena sativa*) is an invaluable nutritional and physiologic cereals and has been much in demand due to its high levels of good quality proteins, lipids, dietary fibers, and non-nutritive bioactive compounds[1]. Oat and its products are used for different purpose in human foods, of which malting can be mentioned. In oat malting certain changes take place, of which the most important occurs to the lipids. Unlike other types of cereals, fat exists throughout the grain that possesses much lipase activity in its neighborhood and its native condition. During germination, the level of free fatty acids (FFAs) rises remarkably and the subsequent oxidation of these acids in storage of malt produces hydroxy acids which result in development of bitter taste [2]. Germination also causes decomposition of carbohydrates and proteins, when glycolytic and proteolytic enzymes are activated. For example starch hydrolyzes slowly and to a little extent during germination [3] and protein hydrolysis lead to the increase of the essential amino acids, lysine and tryptophan [4]. During germination β-glucans are almost entirely decomposed [3] and the great amount of phytic acid is remarkably decreased [5]. There are different strategies to overcome the difficulties in oat malting, one is reducing lipase activity and enhancing α-amylase and proteinase activities during soaking and germination processes. However, to the best of our knowledge, there is no report regarding germination of dehulled oat seed. In this study, the effects of hull and pH in stages of soaking and germination on lipase, α-amylase and proteinase activities were investigated, and then optimized oat malt quality was compared with barley malt.

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II. MATERIALS AND METHODS

A. Materials

Oat (*Avena sativa*) and barley malt were obtained from Iran institute of seed and plant and Behnoush Company in Tehran, respectively. All the chemicals in this study used were purchased from Sigma, Merck and Scharlau companies and were of analytic grade.

B. Methods

1. Chemical Analyses of Oat: Total lipid, protein, fiber, and ash content of the oats were determined using AACC [6] procedures. All determinations were expressed on a dry matter basis.

2. Seed Germination: Grains were used either with or without hull. Dehulling was performed using a laboratory dehuller (OSK, Japan). One hundred grams of the oat was used in each treatment. Having washed the grains with distilled water, they were placed in plastic containers and an equal volume of adjusted solution with pH levels of 3, 4, 5, 6, 7 & 8 was added to each. Hydrochloric acid and sodium hydroxide were used for the adjustment of pHs. Soaking was carried out in two stages and for 16 hours. The grains soaked for 4 hours in the solution, and then aired for 4 hours. In the second stage, the procedure was repeated. Following soaking, the grains were placed in a thin layer on plastic trays. To keep the moisture, paper towels were put both under and over the grains. Germination performed at 16ºC and relative humidity of 100%, wetting of the grains was done 2-3 times a day. Germination ended after 4 days when rootlet length grew *ca*,

1-1.5 cm.

3. Sample Preparation: The samples which were frozen using liquid nitrogen and dried with freeze dryer (Heto-holten, Denmark), were stored at -20ºC until further tests. Prior to the analysis, the samples were hammer milled after removing of rootlets and dehulling (for seeds germinated with hull). To evaluate lipase and α-amylase activities, oat flour with particles passed through the sieve of 40 mesh size was used. To assess the proteinase activity, protein isolate were prepared from the milled grains. In so doing, the milled grains were initially defatted using soxhlet method, mixed with NaOH (0.015N) with the ratio of 1:8 (W:V) and the pH was then adjusted to 9.5 with NaOH. The resulted slurry was stirred for one hour at room temperature, and then centrifuged in 4000×g for 10 minutes. The supernatant solution was neutralized with HCL 2N up to pH 5.5 and was centrifuged again as done previously. Having separated the supernatant, the precipitated part, the oat proteins isolate, was dried using freeze dryer [7].

4. Lipase activity assay: 0.5 ml of the milled sample was mixed with 10 ml distilled water, 2 ml toluene, 1 ml triacetin and 10 ml buffer phosphate (0.1 mol/L, pH 7.6) and was incubated at 38ºC for 24 hours. Subsequently, 100 ml of acetone- ether mixture (3:1) was added to it and was titrated with NaOH 0.1N together using phenolphthalein as an indicator [8].

5. α-amylase activity assay: To determine, the-amylase activity, falling number model 1500 (Perten, Sweden) was used. According to the instruction, for flours with high α-amylase activity, the amount of germinated oat flour for falling 250 was measured, and then it was mixed with substance with no lipase activity up to 7 g. The amount of required germinated oat flour for falling number of 250 about was 0.52% of the enzyme inactivated oat flour, that is equal to 0.0364 g which was mixed with the flour up to 7 g and then the falling number was determined.

6. Proteinase activity assay: Proteinase activity was determined using o-phetaldealdehyd (OPA) method and the degree of protein hydrolysis (DH) was measured [9]. To prepare OPA, 7.26 g di-Na-tetraborat decahydrate and 200 mg Na-dodesyl-sulphate (SDS) were wholly dissolved in deionized water, and then, 160 mg OPA 97% dissolved in 4 ml ethanol was added to the prepared solution. Having added 0.5 ml mercaptoethanol to the solution, the volume was brought up to 200 ml by adding deionized water. This solution was prepared on the analysis day. To analyze, 25 mg oat protein isolate was suspended in one ml of KCL solution (0.1M, pH 1) and was mixed in the vortex for 5 minutes. The solution was then centrifuged in 15700 × g for 5 minutes. 50 µl of the supernatant was added to 250 µl of OPA and after 2 minutes, the absorption was measured in 340 nm wave length the using spectrophotometer (Unico2100, USA).The amount of free amino group was determined with serine standard curve. To prepare the curve, the absorption of the standard solutions containing 20, 40, 60, 80, 100 & 120 µg serine was read in 340 nm and the standard curve was plotted. To calculate the DH hydrolysis, the equation by Nielsen *et al* [10], i.e., $DH= (h/h_{tot}) \times 100$ was used. The h stands for miliequvalent of cleavages peptide bonds in each gram of protein, and h_{tot} represents milieqivalent of peptide bonds in each gram of protein.

7. Malt composition and quality: In this section, chemical analyses and quality of dehulled oat malt that is germinated in pH 5 and barley malt were conducted. Moisture, protein (N×6.25), lipids and ash contents were determined, using AACC Methods [6]. Also, total and reduced sugar contents were determined according to the procedure of AOAC using Lane- Eynon volumetric method [11].

Measuring of hot and cold water extracts, DP and KI were performed, using AOAC procedures [11]. To Measure the amount of cold water extract, ground malt (25 g) was weighed into a beaker and mixed with 500 ml distilled water. The mixture was maintained at 20°C for 2.5 h in the water bath and stirred at 20 min intervals, and then was filtered through Watman No. 1 filter paper and was collected for subsequent analyses.

To determine the amount of hot water extract, ground malt (50 g) was weighed into a beaker and mixed with 200 ml, 46°C distilled Water. The mixture was transferred to water bath, maintained at 65°C for 30 min. and stirred at 250 rpm. The temperature was raised 5 degrees per 5 min, up to 70°C. Afterwards, the mixer was stopped and 100 ml, 70°C distilled water was added to the mixture, and maintained it for 60 min. the mixture was then filtered through Watman No. 1 filter paper. Next, one ml of each hot and cold collected extracts were weighed and the yield of them were determined from the specific gravity (20°C/20°C) equivalent of extract from Plato tables. Diastatic power of the malts was expressed as °IOB. The KI of the malts was calculated by expressing the soluble nitrogen as the percent of total nitrogen in the malt. In so doing, soluble nitrogen was determined using AOAC procedures. Estimations of various parameters were carried.

8. Statistical analysis: The experimental design was randomly performed in duplicate in factorial design. The results were analyzed using ANOVA. Comparison of means of treatments with LSD was done using SAS software ($p<0.05$).

III. RESULTS AND DISCUSSION

A. Chemical Composition of Oat

TABLE I

EFFECTS OF HULL AND pH ON THE ENZYME ACTIVITY OF OAT DURING GERMINATION.

Enzyme	With hull						Without hull					
	pH						pH					
	3	4	5	6	7	8	3	4	5	6	7	8
Lipase[1]	19.2^{cd*}	19.7^{bc}	19.3^{cd}	20.8^{a}	21.0^{a}	20.0^{b}	13.3^{h}	15.3^{g}	15.3^{g}	16.0^{f}	18.8^{de}	18.3^{e}
	±0.07	±0.35	±0.07	±0.35	±0.07	±0.35	±0.07	±0.07	±0.00	±0.35	±0.07	±0.14
α-amylase[2]	273.5^{ab}	271.0^{ab}	267.5^{ab}	263.5^{b}	272.5^{ab}	274.5^{a}	235.5^{cd}	240.5^{c}	217.0^{e}	213.0^{e}	229.6^{d}	243.5^{c}
	±7.78	±3.54	±3.54	±7.07	±10.61	±2.12	±0.07	±2.12	±2.12	±2.83	±4.94	±4.94
Proteinase[3]	12.4^{g}	22.6^{c}	12.7^{h}	18.1^{f}	26.8^{a}	18.3^{b}	13.7^{g}	21.1^{d}	10.4^{i}	12.3^{h}	24.2^{b}	19.1^{e}
	±0.35	±0.42	±0.49	±045	±0.18	±0.25	±0.16	±0.04	±0.23	±0.18	±0.40	±0.42

* Values with similar superscripts in rows do not differ significantly ($p \leq 0.05$).

[1] ml 0.1M NaOH/g

[2] Falling number

[3] Degree of hydrolysis

The results indicated that, assayed oat contains 6.75% lipid, 12.23% crude protein, 6.17% crude fiber and 2.73% crude ash.

C. The Effects of Hull and pH on Lipase Activity

Samples with hulls contained free fatty acids much more than dehulled oats (Table I). As Liukkonen *et al* [12] showed, in soaking stage, lipase had little activity, however, the level of FFAs which was greater in dehulled oats, decreased. The triglycerides level which was also lower in dehulled oats, increased. Peterson [13] revealed that lipase activity became several times higher during germination while total lipids in naked oats declined faster than that of in oat covered with hull. The total lipid and FFAs in some hulled oats increased, which indicate the formation and decomposition of lipids during germination. Such studies showed the critical role of the hull in increased lipase activity. The reason might be the prevention of leaking of lipids and its derivatives by the hull.

The effects of different pH levels on lipase activity were more remarkable among dehulled oats than that of other ones. The FFAs levels increased with the rising of pH from 3 to 7 and decreased with pH rising from 7 to 8 (Table I). Ekestrand *et al* [14] reported that the lipase activity increased significantly in neutral and alkaline pH, which was still more prominent in neutral pH. Lipase activity was lower or constant in acidic pH. The increased lipase activity in neutral and alkaline pH could be due to metabolic processes initiated by the growth of the embryo. Liukkonen *et al* [15] reported that lipase activity is sensitive to alkaline pH above 8 and it subsequently dropped. They showed that the optimum pH for oat lipase is about 7, which is agreement with the present study.

C. The Effects of Hull and pH on α-Amylase Activity

Alpha amylase activity of germinated oats based on falling number is demonstrated in Table I. Accordingly, the falling number of hulled samples was significantly lower than that of dehulled oats. This might be due to inhibitory effects of hulls on germination, because of high level of moisture of grain during germination, which in turn has a destructive effect on α-amylase synthesis [16]. The difference between falling numbers in various pH levels was greater in dehulled samples than those of hulled ones. The lowest falling number was observed in pH levels of 5, 6 and the falling number were at highest level in high or low pHs. Peterson [3] showed that germination of oats leads to the increase of α-amylase activity up to corresponding level in barley. Kneen [17] demonstrated the low β-amylase activity of oat disappears during germination. Therefore, concluded that oat malt is a rich source of α-amylase. Bertoft *et al* [18] reported that α-amylase became irreversibly inactive in extreme pH levels. Meredit [19] inactivated α-amylase by acidifying wheat flour slurry up to pH≤ 2.5. Beta *et al* [20] suggested that acids cause the reduction of sorghum germination through the prevention of α- amylase synthesis. Tkachuk *et al* [21] compared the various isoenzymes of wheat malt α- amylase in different pH levels and concluded the optimum pH for α-amylase activity is within the range of 5.5-6.6. In the present study, the pH level for germinated oat α-amylase was found to be within the same range (Table I).

TABLE II
CHEMICAL COMPOSITION AND QUALITY OF OPTIMIZED OAT AND BARLEY MALT (CONTROL).

Malt type	Moisture (%)	Ash (%)	Lipid (%)	Soluble N (%)	Total N (%)	Kolbach index	Diastatic power (°IOB)	Hot water extract (%)	Cold water extract (%)
Oat	5.55^{*a} ±0.01	2.35^{b} ±0.01	7.1^{a} ±0.01	0.704^{a} ±0.002	2.92^{a} ±0.13	23.72^{b} ±0.06	62.6^{b} ±2.12	107.01^{b} ±0.78	10.35^{b} ±0.03
Barley	4.89^{a} ±0.02	2.44^{a} ±0.01	3.7^{b} ±0.01	0.606^{b} ±0.002	1.83^{b} ±0.15	37.85^{a} ±0.03	81.5^{a} ±2.17	137.75^{a} ±0.75	22.34^{a} ±0.05

* Values with similar superscripts in rows do not differ significantly ($p \leq 0.05$).

D. The Effects of Hull and pH on Proteinase Activity

Germinated oat proteinase activity based on the degree of hydrolysis was shown in Table I. Samples with hull had greater proteinase activity than the dehulled ones. The high degree of protein hydrolysis in hulled oats might be duo to the prevention of moisture loss and consequently the remaining high level of it in the grains. This gives rise to increased protein hydrolysis. All the oat samples had greatest protein hydrolysis in pH levels of 4 and 7 and lowest in levels of 3 and 5 (Table I). Zhang *et al* [22] reported that the most endoproteinase of barley are active in pH levels lower than neutral. Active predominant barley proteinase in low pH levels (3.8-4.4) were cysteine and aspartic acid proteinases. Mikola *et al* [23] suggested that high activities of these enzymes in low pH (3.8) arise from the ability of aleuron layers to acidify its surrounding environmental and it seems that oat aleuron lacks this capability. They also demonstrated in another study that the onset of proteinase activity is the same during germination of both oat and barley. Four day after germination, serine and metalloproteinases were predominant in oats. Despite the activity of these enzymes in pH 6.2 (endosperm pH of germinated oats), little hydrolysis by them take place in low pH. There might be compartments or regions within endosperm where pH levels are so low that cysteine proteinase become active to hydrolyze globuline and avenin [24]-[25]. Proteinase activity decreased in extremely acidic pH [20]. Low proteinase activity in pHs 3 and 5 might be duo to the effects of the corresponding solutions on the pH of the region within endosperm, and changing them so that the condition would be inappropriate for cysteine proteinases. Meanwhile, the role of serine and metalloproteinases in protein hydrolysis is not that much of important and the most activities of them are reported to be in pH range of 6.0-8.5. Therefore, we can conclude that high proteinase activity in pH 7 is not caused by the above mentioned enzymes.

E. Malt Composition and Quality

Certain chemical properties and quality parameters of dehulled oat malt that is germinated in pH 5 and hulled barley (as control), are shown in Table II. The results indicate that the moisture contents in oat and barley malts are not significantly different. While the amount of ash in oat malt was significantly less than that in barley malt, which might be due to the less amount of minerals in oat. The amounts of minerals in the barley and oat hulls are more than dehulled grain. Also, the amount of minerals in whole grain of barley is more than that in oat. Thus, the higher amount of ash in barley malt is confirmed [26].

Comparing the means in Table 2 shows that the amount of total and soluble nitrogen in oat malt is significantly more than that in barley malt that may be because of greater content of protein in oat. On average, different oat varieties compared to barley contain higher amount of protein, which is 8-20% and 10-16% in Oat and barley, respectively based on dry weight [26].Germination and malting cause increase in proteins contents of the grain. By degradation of storage proteins, the amounts of soluble protein i.e., albumin, are increased and the amounts of other proteins are reduced [27]. More than 70% of the proteins in barley malt degrade during malting [28].Total protein content cannot be merely the suitable index for determining the malt quality, hence, the proportion of soluble protein to total protein, i.e., Kolbach index, should be calculated, as well. The greater the total protein amount, the lower the Kolbach index. Proper range of KI in beer making is 38-42. Within this range, the sufficient amounts of low and high molecular weight proteins enter the drinks [29]. In the present study, KI for oat malt was lower than that for barley with less protein, which may be because of the greater protein content in oat (Table II). This result is agreement with Pomeranz [30] study.

Barley malt has significantly greater DP than oat that may be due to more diastase activity of barley compared to oat. More than 99% of starch degradation is related to the alpha and beta amylase enzymes. If proteolysis suffices, adequate amounts of alpha-amylase are produced and then the DP can increase [31]. Davidson [32] and Pomeranz [30] also showed that the barley has greater DP than oat.

The hot and cold extraction yield of oat malt was significantly less than that of barley that may be caused by less diastase and proteinase activities of oat malt relative to barley (Table II). Cold water extraction indicates the changes in the protein and carbohydrate during germination and malting. At this stage, proteins and carbohydrates break down

and the sugars and amino acid can enter the extract. Hot water extraction represents the amount of soluble solids that entering the extract from malt and adjuncts during mashing. Surveys show that malts with higher KI have a greater extraction yield. Accordingly, protein modifications should be balanced with carbohydrate changes as well [28]. Higher extract yield of barley malt compared to oat has also been reported by Pomeranz [30] and that is confirmed the obtained results.

Lipid content obtained in oat malt was significantly greater than that in barley which might be due to the greater amount of fat in oat. Normally, the barley and dehulled oat have 1-3% and 4-6% fat content, respectively. Considering the small amount of fat in the hull, the amount of fat in the dehulled grains is approximately 5-10% or on average 7% [26]. Presence of high fat content in oat malt not only increases the turbidity of the extract and beer, but also destabilizes the foam and taste of drinks during storage, which damage the product quality [33]-[34]. Therefore, high lipid content in malt must be considered as a defect and appropriate methods are needed to reduce its content in grain, extract or drinks.

IV. CONCLUSION

During the germination of oat, hull serves as a physical barrier and prevents losing of moisture, lipid, protein, carbohydrate and their derivatives from the seed. It may leave either positive or negative impacts on enzyme activities, with negative effects more on oat enzyme activities. Also, pH levels of 4 and 5 had more positive effects on oat enzyme activities, with pH 5 more prominent. Therefore, dehulled samples treated with pH 5 are suitable for malt making. Nevertheless, barley malt, as demonstrated in this study, possesses better qualities than optimized oat malt for beer making.

ACKNOWLEDGMENT

The authors would like to acknowledge the Isfahan University of Technology for financial support of this research and B. Bahrami for technical assistance. Our thanks are due to H. Khajehei for his help with linguistic copy editing.

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