Optimization of Fermentation Time for Iranian Black Tea Production

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ABSTRACT: The optimum fermentation times of black tea manufactured by two systems of Orthodox and CTC (cut, tear & curl) were investigated by measuring the quality parameters of black tea, like: theaflavin, thearubigin, highly polymerized substances and total liquid color during the fermentation stage. Optimum fermentation times from the beginning of fermentation were determined to be 60 min and 150 min for the fine and coarse tea of orthodox manufacturing, respectively. The optimum fermentation time of CTC tea process was 30 min. Most theaflavin content of Orthodox and CTC processes were 0.63 and 0.82 percent of the total dried solids of infusions, respectively. Total color of CTC manufacturing was 16% higher than Orthodox process.

KEY WORDS: Iranian black tea, Orthodox, CTC, Theaflavin, Thearubigin, Optimum time, Fermentation.

INTRODUCTION
Black tea beverage made from green tea leaves of 'Camellia sinensis' is the most popular national drink in Iran. Annual production of black tea is about 61000 ton in Guilan province covering more than 34000 ha of tea farms as the major tea manufacturing region in Iran. The use of a Chinese variety hybrid along with traditional methods of orthodox rolling (ORTH) is the common practice for tea processing in Iran, involving withering, rolling, sieving, fermentation, drying and sorting [1].

Fermentation is the critical stage in black tea manufacturing. The endogenous flavanols in the tea leaves undergo various oxidative enzymatic reactions. Catechins and their gallates, the major components of the polyphenolic fractions of the green tea leaves are the substrates of poly phenol oxidase [PPO; EC 1.14.18.1; monophenol mono-oxygenase or EC 1.10.3.2; o-diphenol: O2 oxidoreductase] which is the key enzyme and has a significant role in formation of the black tea components: theaflavins (TFs) and thearubigins (TRs) [2, 3, 4].

The influential factors in these enzymatic reactions, in addition to physical and genetic properties of tea leaves, withering and rolling conditions are: moisture, temperature, O2 availability and the fermentation time. During black tea manufacturing, the TF: TR ratio is changes with various fermentation times [3, 6]. The best TF:TR ratio is considered to be 1:10 by Owuor (1986). With respect to the strong correlation between quality of black tea and the amount of colored TF product, the TF...
value of the black tea is proposed to be a useful measurable parameter for the tea quality evaluation [2, 5 and 6].

Visual assessment of 'dhool' (macerated tea leaves) by factory workers is the common procedure to determine optimum fermentation time in Iranian tea factories. Moreover, in recent years some factories have changed from conventional tea processing to cut-tear-curl (CTC) method. So, it is important to determine the optimum fermentation time in both processing methods.

The present study describes the optimization of fermentation time of green leaves of Iranian tea plants processed by CTC and the orthodox methods.

MATERIALS AND METHODS

Leaf Plucking

Tea clone representing ‘clone 100’ of Chinese variety was selected for the study from ‘Fashalam’ tea plantation and research institute of ‘Guilan’ province, situated at altitude of 7 m below the sea level, latitude 37º 25” N and longitude 49º 25” E, while plants receiving 370 Kg N ha⁻¹ year⁻¹ as NPKS 15:1:7:3 compound fertilizer in a single dose 3 months prior to experiments.

Leaf manufacturing

The apical bud and two leaves were hand plucked in the middle of may and then withered for 15 h until it losses 30% and 40% of its structural moisture content for CTC and ORTH process, respectively.

For CTC manufacturing, withered leaves made into cut dhool thrice by an experimental CTC machine model 3 inch bench teacraft, and dhool sieved with 10 meshes Std. Sieve no. into small particles.

For ORTH manufacturing, withered leaves rolled with an experimental machine for 1 hour.

During fermentation period 400-900 gr dhool was used (CTC, ORTH) and manufacturing was carried out in triplicate with CTC and ORTH processes. Macerated leaves were transferred into a fermentation room under controlled temperature of 25°C and 90% saturation humidity. For 30 min intervals, 80 gr. of dhool was taken and dried with a ‘tea craft’ device at 105°C until moisture content was reduced to less than 4%. The fired tea was taken into plastic packs and stored in a dry place.

Chemical Analysis

Quality parameters of samples: TF, TR, high polymerized substances (HPS) and total color (TC) were evaluated by solvent extraction method of Angayarkanni (2002) which is depicted in Fig. 1 and briefly described as below:

4 gr. of dry tea was extracted in 200 ml of boiling water for 10 min. 1 ml of filtered infusion was eluted with 9 ml of distilled water and its absorbance on $\lambda=460$ nm measured with a Perkin Elmer Lambola 3A UV/VIS spectrophotometer and was considered to be as A.

25 ml of infused tea was added to 25 ml of iso-butyl methyl ketone (IBMK) and after sufficient mixing, two phases allowed to be separated. 1 ml of organic phase was eluted with 9 ml of ethanol (45%) and its absorbance at 380 nm was considered to be as B.

10 ml of organic phase and 10 ml of Na₂HPO₄ (2.5%) were mixed together and allowed to be separated into two phases.

The aqueous layer was discarded and 1 ml of organic layer was diluted with 9 ml of ethanol (45%). Its absorbance at 380 nm was considered to be, C.

10 ml of the first aqueous phase was mixed with 10 ml of n-butanol and allowed to be separated into two phases. 1 ml of this organic layer was eluted with 9 ml of ethanol and its absorbance on $\lambda=380$ nm was considered as D.

1 ml of the aqueous layer was eluted with 9 ml of ethanol and its absorbance at 380 nm was considered as parameter E.

Amounts of quality parameters of tea samples were determined using the following formula, which was proposed by Angayarkanni [7]:

$\text{TF} (%) = 4.313 \times C$

$\text{TR} (%) = 13.643 \times (B+D-C)$

$\text{HPS} (%) = 13.643 \times E$

$\text{TC} (%) = 10 \times A$

The multiplication factors mentioned in the equations were derived from molar extinction coefficients of pure compounds and the dilution factors [7]. HPS was represented as TR. In case of TC the dilution factor was 10.

RESULTS AND DISCUSSION

Fermentation stage, at which the tea samples were taken for quality parameters determination of the both
The TF content of dhool increased during tea fermentation in the CTC method and after 30 min declined with time, while the TR and HPS contents revealed a rising pattern (Fig. 2).

Optimum fermentation time of the CTC tea manufacturing was determined to be 30 min with 0.82 percent of total dried solids of infusion. Lower TF content was obtained at longer oxidation times, due to transformation of TF into TR and HPS groups [3, 12].

Theaflavin content of clone 100 of Iran, processed via CTC method reached its peak faster than of clones 6/8 and S15/10 of Kenya, as reported by Owuor (1994 & 1998) and Obanda (2004), whose theaflavin peaks occur after 147 and 90 min, respectively [5,10,11].

Total color of the CTC tea approached plateau after 1 hour fermentation with 3.03 percent color pigments of the initial black tea weight (Fig. 2). In fact the differences in total color levels due to an increase in fermentation time after 30 min were not significant, but with the increase of fermentation, the quality of tea declines because of reductions in TF content and TF:TR ratio, resulted in less brightness of black tea liquor (Figs. 2 and 4), [11, 13].

The pattern of TF production in ORTH processing show two peaks in 60 and 150 min of fermentation because of different sizes of macerated leaves formed during rolling (Fig. 3).
Table 1: Comparison of quality parameters of some teas.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Clone</th>
<th>process</th>
<th>TF (%)</th>
<th>FR (%)</th>
<th>HPS (%)</th>
<th>TC (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese</td>
<td>IR-100</td>
<td>ORTH</td>
<td>0.35</td>
<td>9.1</td>
<td>11.3</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>IR-100</td>
<td>ORTH</td>
<td>0.59</td>
<td>10.8</td>
<td>10.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Chinese</td>
<td>IR-100</td>
<td>CTC</td>
<td>0.8</td>
<td>7.6</td>
<td>8.4</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Assam</td>
<td>UPASI-3</td>
<td>ORTH</td>
<td>1.05</td>
<td>9</td>
<td>8.28</td>
<td>3.12</td>
<td>6</td>
</tr>
<tr>
<td>Chinese</td>
<td>UPASI-9</td>
<td>CTC</td>
<td>0.78</td>
<td>7.6</td>
<td>7.1</td>
<td>2.6</td>
<td>8</td>
</tr>
</tbody>
</table>

a) Tea of a conventional factory  
b) Iran ORTH manufactured tea with 150 min fermentation  
c) Iran CTC manufactured tea with 30 min fermentation

Fig. 2: pattern of changes in quality parameters in the CTC black tea manufacturing; percents of TF, TR and HPS are percents of dry infusion of total color pigments; percent of TC in the percent of dry black tea weight.

Upon analysis of the TF content, it appears that the optimum fermentation times in ORTH system be 60 min for fine tea (that is separated in primary stage of rolling by sieving in factories), and 150 min for coarse tea (that remains on the sieves).

The HPS production increased during dhool fermentation and had a similarity to the pattern of the TC production (Fig. 3). So, it appears that the role of the HPS in the color production of ORTH teas is more important than other TR groups.

The total color of ORTH made tea increased slowly during fermentation (Fig. 3). The total color of CTC manufacturing was 16% higher than ORTH process at their maximum, (3.03 and 2.6%, respectively).

The TF content of CTC process was 26% higher than that of the ORTH at the optimum time of fermentation, (0.80 and 0.59%, respectively). The CTC system improves black tea quality because of the more effective exposure of the endogenous enzymes and polyphenols to the oxygen resulted in better breaking down of cellular structure [2].

The TF:TR ratio in CTC process approaches to 0.1 after 30 min fermentation. It confirms that 30 min after maceration process is the optimum fermentation time for clone 100 of Iranian tea under CTC manufacturing (Fig. 4).

The TF:TR ratio approaches to 0.1 after 180 min fermentation time in the ORTH process, while this ratio approaches to 0.08 and 0.06 after 60 min and 150 min fermentation in the ORTH process, respectively. The amount of TF content and the TF:TR ratio confirms that 60 min is the best fermentation time for fine tea leaves.
the manufacturing of tea under accurate conditions results in better quality of black tea production.

For manufacturing of high quality black tea, having a high quality green leaf is the first prerequisite. Iranian tea manufacturers try to improve their manufacturing methods to achieve the best quality. But, they should consider that the flavanols contents of variety Assamica are two fold higher than of variety sinensis [8], so under the same manufacturing process, the TF content of black tea from variety Assamica always exceeds the TF content of the black tea the produced by sinensis variety (Table 1).

CONCLUSIONS

TR could form through many pathways during fermentation, so there are various groups of TR [3]. Heterogeneous shapes and sizes of rolled leaves with some limiting oxygen availability conditions possibly cause fluctuations in ORTH manufacturing. Also these conditions lead to higher HPS concentration than TF molecules (Fig. 3).

The formation of balck tea polyphenols during fermentation may be considered of two reaction (Fig. 5). The initial reaction, catalyzed by PPO, is responsible for the oxidation of catechins to their o-quinones. In the subsequent reactions, o-quinones condense to form TFI and other undefined pigmented compounds such as TRs. Also, gallocatechin quinines are able to react together to form TR groups.

At the start of fermentation, formation of TF and TR groups is competitive, but on later stages some of TF groups undergo condensation and formation of TR complexes. Robertson (1983) concluded that oxygen concentration had the most important role in the formation of TF molecules or TR groups from TF intermediates. Composition of the TR fraction of the ORTH rolling may be very different from those produced by more efficient cell disruption processes. Comparison of the ORTH and CTC processes show more HPS groups produced by the former (Figs. 2 and 3). Therefore liquors of the ORTH manufactured tea are less bright than that of the CTC manufacturing [4, 8].

Today, the Iranian black tea market is smaller than the Chinese variety of Indian teas, because of some inadequate plucking of green leaves (coarse) and considering only quantity of manufacturing. With respect to standard plucking (Bud & 2) and use of optimum process
condition, Iranian tea can find its real position in the tea world ranking.

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