Abstract

India produces nearly the world’s entire turmeric crop with its inherent qualities and high content of important bioactive compound like curcumin, which is a proven potent antioxidant. Alongside India produces 65-75 million tonnes of wheat annually which is mostly consumed by the Indians in form of wheat flour (aata). Nutritionally rich wheat flour degrades totally within forty five days and become unfit for human consumption. The present study investigates the antioxidant and also the antimicrobial activity of curcumin when impregnated on wheat flour under normal home storage conditions. After five weeks it was observed that the degradation of starch can be lowered upto 29% and that of gluten upto 20% on impregnating wheat flour with 0.30% curcumin.

Keywords: antioxidant , Curcumin, degradation, gluten, starch, wheat flour

Introduction

Turmeric is a plant that has a very long history of medicinal use, dating back nearly 4000 years. Because of its brilliant yellow color, turmeric is also known as “Indian saffron.” The reported consumption of turmeric in Asian countries in humans is in the range of 200–1000 mg/day [1] or 160–440 g/person/year [2]. Discovery of its active constituent-“curcumin” has considerably changed the significance of turmeric and it is looked as a medicinal treasure [3]. Out of the 100 components isolated from turmeric the main components are turmerone, and curcuminoids. Curcuminoids consist of curcumin demethoxycurcumin, 5’-methoxycurcumin, and dihydrocurcumin, which are found to be natural antioxidants [4,5]. Curcumin is a low molecular weight polyphenol is regarded as the most active constituent of turmeric. It has been the subject of hundreds of published papers studying its antioxidant, anti-inflammatory, cancer chemopreventive and potentially chemotherapeutic properties [6,7]. The great potential of curcumin is due to the fact that it acts upon several important molecular targets and can be used to treat various disorders [8]. According to Khopde et al. [9] curcumin is at least ten times more active as an antioxidant than even vitamin E. Curcumin is known to protect bio-membranes against peroxidative damage by scavenging the free radicals involved in peroxidation. The antioxidant mechanism of curcumin may be due to the scavenging or neutralizing free radicals, inhibition of oxidative enzymes, oxygen quenching etc. [10, 11]. Priyadarshini et al. [12] have concluded that phenolic group is essential for the free-radical scavenging activity and the methoxy group further increased the activity. Curcumin also inhibits the growth of varieties of microbes such as viruses, bacteria and some pathogenic fungi [13].

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After rice wheat is the most important cereal in India. People in India consume 70-72 million tonnes of wheat on an average. In India, it is commonly known as 'atta' and is consumed mostly in the form of rotis, chapatis, etc. Wheat grains have the basic structure made up of a germ, endosperm, and bran. The germ is of importance with respect to food quality as it has high "oil" content. The fatty acid content of the germ oil is generally very susceptible to oxidative rancidity, particularly once the germ has been "cracked" with milling. The shorter shelf life of whole wheat flour, as opposed to all-purpose flour, is attributed to this portion of the grain. The nutrient rich wheat is only grain with high gluten content, which attributes to 75-80% of protein. The starch of wheat consists of amylose and amylopectin which are also susceptible to degradation like the protein of wheat flour during usual storage due to various factors like atmospheric moisture, enzymatic decay, oxidative breakdown and microbial infestation.

Thus, the present work evaluates the antioxidant and antimicrobial activity of curcumin on wheat flour by impregnating wheat of two varieties (loose wheat flour available in the market and packaged flour of a reputed brand) with varied proportions of curcumin and to study whether addition of curcumin could lessen the degradation of wheat starch and gluten when the mixtures are being kept at the normal home storage condition.

**Experimental**

**Materials and Reagents**
Whole wheat flour (Branded packaged & Non branded-loose) purchased from local market of Kolkata, India, Curcumin (AR grade) purchased from Central Drug House Pvt. Ltd., India, Diethyl ether & ethyl alcohol, Conc.HCL, Methylene blue indicator, Fehlings I & Fehlings II solution-all solvents and chemicals used were obtained from M/s. E-Merk (India) Ltd.

**Estimation of starch content**
0.001kg of sample wheat flour was washed with diethyl ether and 90% ethyl alcohol for complete removal of curcumin from the sample. The washed flour was treated with 0.02 litre of conc. HCL and kept overnight. Next day, the sample solution was boiled in a water bath for 4 hours & filtered. The filtrate was titrated by Fehling’s solution using methylene blue indicator.

**Estimation of Gluten Content**
0.010 kg of sample wheat flour was mixed with little water to produce a dough. Then the dough was allowed to stand for 1 hour. The dough was then put in a piece of cloth and washed in a stream of cold water until the starch and all soluble matters were totally removed. Then the gluten was collected quantitatively from the cloth, spread in a petridish and dried at 105°C for 4 to 6 hours in hot air oven. Then it was cooled in a dessicator and weighed.

**Plate count method for mould growth**
The wheat flour samples were dissolved in 0.1litre of distilled water. The sterilised CZAPECK DOX agar medium was poured into petridishes and cooled. The sample was serially diluted following standard procedure and poured into the petridish with CZAPECK DOX agar medium and incubated for 5 days to observe the colony formation.

**Results and Discussion**
Initially a study was conducted for five weeks to observe the effect of addition of 0.06, 0.12, 0.18, 0.24 & 0.30 g of curcumin to 100 g of loose wheat flour available in the local market of Kolkata. The results are depicted in the Table 1 which shows the degradation of the starch content in wheat flour in presence of different amount of curcumin. It was observed that throughout the 5 week study 0.06% and 0.12% addition of curcumin does not reflect any appreciable change. Inhibition of degradation of starch is significant when curcumin addition is increased to 0.18% and it was maximum at 0.30%. Presence of 0.30% curcumin in the loose wheat flour could protect and retain the starch content as high as 64.7% even after 5 weeks of storage under normal conditions when in comparison the normal loose wheat flour contained only 40.4% of starch under similar conditions. Further study is continued with
18% and 30% of curcumin addition. Curcumin addition is not increased further as more curcumin may produce a bitter taste in the flour and also changes the colour which not at all desirable. As wheat flour is consumed both in loose and packaged form the study was also conducted with branded wheat flour. While packaged flour normally containing 64.4% of starch was found to contain only 42% of starch after 5 weeks of normal storage which is considerably higher on addition of 0.18% and 0.30% curcumin and the values are 53.7% and 57.4% of starch respectively (Table 2).

Along with starch, Gluten is also an important component of wheat flour. Impregnation of whole wheat flour with different proportions of curcumin also protects the degradation of Gluten both in loose and packaged flour as indicated by Figure 1 & 2. Loose wheat flour initially containing 14.01% can retain 8% of Gluten and packaged wheat flour containing 12.7% of Gluten can retain 8.2% after 5 weeks of normal storage. Thus without any curcumin the overall degradation of starch and gluten was as high as about 49.7% and 6.1% in loose wheat flour. Whereas on addition of 0.30% curcumin on the same wheat flour sample the degradation is considerably lowered, i.e. 25.4% for starch and 4.3% for gluten. Degradation is also retarded with the impregnation of 0.18% curcumin both in loose and packaged wheat flour as evidenced from Table 3.

The microbiological study of the mixtures of wheat: curcumin has also been undertaken as indicated in Figure 3 and Figure 4. The experimental fact revealed that flour mixture having wheat:curcumin ratio of 100:0.30 showed considerable lesser growth of mould by at least 44%, followed by 25% (100 : 0.18) when compared to the blank sample (100 : 0) when the wheat flour sample is branded and packaged. Similarly in case of loose wheat flour mould growth was restricted around 36% and 20% when wheat:curcumin ratio is 100:0.30 and 100: 0.18 respectively. Thus the potential antioxidant; curcumin can also exert its antimicrobial activity regarding the storage of wheat flour.

Thus, it may be concluded that curcumin truly exerts its antioxidant activity on the deteriorating factors that are responsible for the degradation of normal home storage of wheat, especially in tropical countries where the effect of ambient moisture, oxidative effect and microbial infestations come into play with great vigour and triumph.

The study was also further stretched to get a graphical analysis to predict the zero degradation or negligible degradation of wheat flour with respect to starch and gluten during storage of five weeks. From the Figure 5, zero degradation for gluten was found to be at the wheat: curcumin ratio of 100: 1.094 whereas for starch (Figure 6), zero degradation will be at wheat: curcumin :: 100: 0.624. Thus the graphical extrapolation suggest that impregnation of 1.09% curcumin to the normal wheat flour may protect the wheat flour at least upto 5 weeks of home storage without any degradation or negligible degradation with respect to starch and gluten.

**Table 1** Effect of curcumin on stability of starch in wheat flour (loose)

<table>
<thead>
<tr>
<th>Curcumin (%)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>90±0.02</td>
<td>87.8±0.02</td>
<td>74.0±0.02</td>
<td>68.1±0.02</td>
<td>59.9±0.02</td>
<td>40.4±0.02</td>
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<tr>
<td>0.06</td>
<td>87.7±0.02</td>
<td>74.1±0.02</td>
<td>68.1±0.02</td>
<td>60.1±0.02</td>
<td>40.6±0.02</td>
<td></td>
</tr>
<tr>
<td>0.12</td>
<td>87.9±0.02</td>
<td>74.4±0.02</td>
<td>69.3±0.02</td>
<td>60.7±0.02</td>
<td>40.9±0.02</td>
<td></td>
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<tr>
<td>0.18</td>
<td>88.2±0.02</td>
<td>80.8±0.02</td>
<td>73.3±0.02</td>
<td>65.7±0.02</td>
<td>53.4±0.02</td>
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<tr>
<td>0.24</td>
<td>90.0±0.02</td>
<td>83.9±0.02</td>
<td>78.1±0.02</td>
<td>71.6±0.02</td>
<td>63.2±0.02</td>
<td></td>
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<tr>
<td>0.30</td>
<td>90.1±0.02</td>
<td>84.2±0.02</td>
<td>79.5±0.02</td>
<td>73.0±0.02</td>
<td>64.7±0.02</td>
<td></td>
</tr>
</tbody>
</table>

*Values are reported as mean ± standard error, where n=3*
Table 2 Effect of curcumin on stability of starch in wheat flour (packaged)

<table>
<thead>
<tr>
<th>Curcumin (%)</th>
<th>No. of Weeks</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>0.0</td>
<td>64.4±0.08</td>
<td>57.5±0.14</td>
<td>55.3±0.11</td>
<td>51.3±0.05</td>
<td>46.8±0.07</td>
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<tr>
<td>0.18</td>
<td>62.4±0.07</td>
<td>59.7±0.12</td>
<td>57.0±0.13</td>
<td>55.5±0.12</td>
<td>53.7±0.02</td>
</tr>
<tr>
<td>0.30</td>
<td>63.7±0.10</td>
<td>61.9±0.06</td>
<td>59.7±0.07</td>
<td>58.5±0.06</td>
<td>57.4±0.06</td>
</tr>
</tbody>
</table>

Values are reported as mean ± standard error, where n=3

Table 3 Overall degradation of wheat flour starch and gluten in 5 weeks on impregnation with different proportions of curcumin

<table>
<thead>
<tr>
<th>Curcumin (%)</th>
<th>Starch</th>
<th>Gluten</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Loose</td>
<td>Packaged</td>
</tr>
<tr>
<td>0</td>
<td>49.7±0.11</td>
<td>22.3±0.10</td>
</tr>
<tr>
<td>0.18</td>
<td>36.7±0.09</td>
<td>10.7±0.07</td>
</tr>
<tr>
<td>0.30</td>
<td>25.4±0.07</td>
<td>7.0±0.11</td>
</tr>
</tbody>
</table>

Values are reported as mean ± standard error, where n=3,

Figure 1 Effect of curcumin on stability of Gluten in wheat flour (loose)
**Figure 2** Effect of curcumin on stability of gluten in wheat flour (packaged)

**Figure 3** Microbial study on packaged wheat flour during home storage conditions

**Figure 4** Microbial study on loose wheat flour during home storage conditions
Conclusion

Our present clearly indicates that curcumin, which is a proven antioxidant can also exert its antioxidant and antimicrobial properties for protecting the wheat flour during its storage under normal conditions. The study revealed that starch degradation could be arrested by at least 49% and gluten by at least 28.3% when 0.30% of curcumin is mixed with whole wheat flour available in the market. The novel antioxidant also exerts its antimicrobial property and could resist the growth of mould by at least 44% and 25% when 0.30% and 0.18% curcumin was impregnated on wheat flour in comparison to the flour sample without any curcumin addition. Thus, by impregnating a cereal like wheat with a potent antioxidant curcumin, which is the staple food consumed by a considerable section of the world, the deteriorating or damaging effects of our natural ambience during normal home storage conditions, especially, in the tropical countries, the degradation with respect to starch and gluten may be reduced considerably or inhibited at certain ratio of wheat : curcumin. The study is further being continued to observe the beneficial effects of the other components of turmeric, so that in future turmeric fortified wheat flour may be produced which possibly will be an important functional food of our regular diet.
References


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