Stabilization of fat degradation process in wheat germ (*Triticum spp*) for edible purposes

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Abstract **- Scope of this study is to investigate the factors responsible for the fat degradation process of wheat germ and to determine preventive measures according to factorial experimental design. Therein, 10kg of wheat germ was taken (M.C 11.63%, Acid value 14.92mgKOH/1g oil) and divided into two portions. One portion was steam blanched at 110^{o}C for 3 minutes and the rest portion was kept untreated. Blanched portion was divided into two, and moisture content of one portion was reduced to 8% (w/b) while keeping the rest portion untreated. These two portions were divided into two again and one portion of each was kept at refrigerator condition (-18^{o}C) and the rest two kept at in house condition. The same procedure was followed for un-blanched portion too. Samples were drawn from each treatment biweekly up to 6 weeks and subjected to determine acid value. All treatments were triplicate and data were analyzed statistically to determine the best treatment. Finally, acid profile of best treatment was compared against same of fresh sample using GC/MS. Results revealed that best treatment was blanched and low moisture sample, stored either in refrigerated or in-house condition. Acid values of these two treatments after 6 weeks of storage were 20.12 & 20.50 respectively as against initial value of 14.92. Fatty acid profiles of fresh and best treatment (Linoleic acid, Linolenic acid, Oleic acid, Capric acid, Lauric acid, Myristic acid, and Stearic acid) were 58.46%,7.96%,14.32%, 0.024%, 0.031%, 0.116%, 17.936% & 0.508% and 56.62%, 7.12%, 15.46%, 0.029%, 0.024%, 0.104 & 0.496% respectively.**

Index Terms**- wheat germ, tocopherol, oleic acid, linolenic acid, linoleic acid, acid value**

I. INTRODUCTION

In wheat milling process usually germ is removed although it is rich in protein, B vitamins, minerals and fats. In contrast to its nutritional potential, wheat germ has a very poor storage quality and this is the major constraint in it being separated and utilized for augmenting the supply of nutritious foods. This is due to very high activities of enzymes as well as large amount of unsaturated fatty acids present in germ (Pomeranz, 1988). Currently the wheat germ is added to animal feed in many wheat milling industries regardless of its nutritional value. Rapid rancidity of wheat germ affects the shelf life of final flour which restricts the addition of wheat germ to flour and storing capability of wheat germ. Wheat germ lipids largely consist of fatty acids (FA). The majority of FA is triglycerides (57%); the most abundant is linoleic acid (18:2), which accounts for 42–59% of the total triglycerides, followed by palmitic acid (16:0) and oleic acid (16:1). The unsaturated fatty acids account for about 80% of triglycerides (Kahlon 1989; Hidalgo et al. 2009). Wheat germ oil (WGO) contains the highest tocopherol content among all other vegetable oils. The majority of the tocopherols in WGO were in the form of α-tocopherol (90% of the total tocopherols) and β-Tocopherol was the second most abundant tocopherol in the WGO samples (Wang and Johnson 2001).

Therefore aim of this study is to investigate the factors responsible for fat degradation process in wheat germ and possible remedial measures in order to arrest these adverse consequences.

II. MATERIALS AND METHOD

Materials

Fresh wheat germ of Australian soft wheat, moisture analyzer, deep freezer, soxhlet extraction apparatus, rotary evaporator, drying oven, GCMS (Aligent Technologies, 7890A GC system & 59756 inert XL1/01 MSD MS), Analytical balance (Axis

AG220C), Magnetic stir (Microsil, India), Thermometer, Water bath (Microsil, India), Chemicals (AR, Sigma, Fisher, UK) and glassware.

Methodology

Experimental design

This study was conducted according to two factor factorial experiment design, using 3 variables at two levels namely heat treatment (blanched & un-blanched), reduction of moisture content (14% & 8%) and storage condition (refrigerated & in house condition). About 10kg of wheat germ was taken and divided into two portions. One portion was steam blanched at 110^{o}C for 3 minutes and the rest portion was kept untreated. Blanched portion was divided into two again and moisture content of one portion was reduced to 8% (w/b) while keeping the rest at 14%. These two portions were divided into two again and one portion of each was kept at refrigerator condition (-18^{o}C) and the rest two portions were kept at in house condition (RH: 68-72%, Temp: 26-28^{o}C). Same procedure was followed for un-blanched portion of wheat germ too. The experiment design of the study is shown in figure 1.

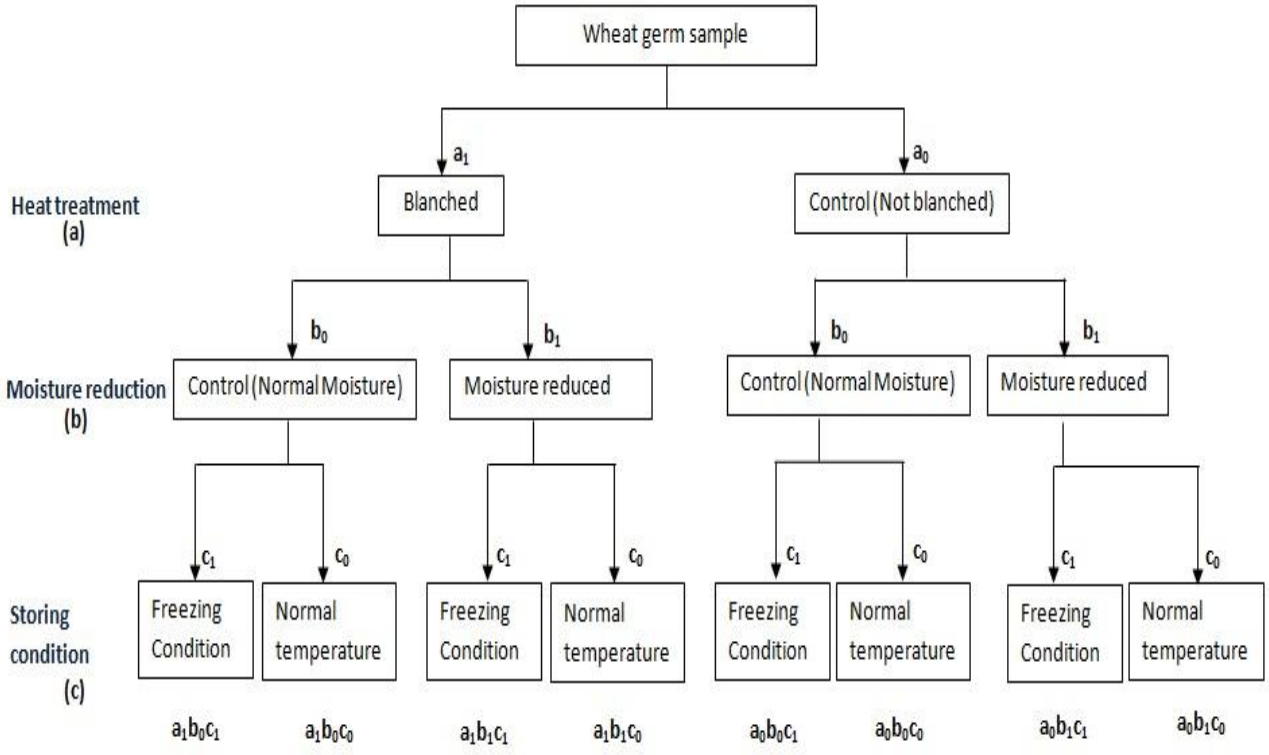

Fig. 1 Experimental design

Samples were drawn from each treatment biweekly and subjected to oil extraction using soxhlet apparatus and extracted oils were used to analyze the acid value according to AOAC Official method, 1984, 996.01.

Determination of moisture content in wheat germ

Moisture content of fresh wheat germ was measured according to AOAC Air oven method 925.10 using an instant moisture analyzer (AnD MX-50).

Sample preparation for GCMS analysis (preparation of fatty acids methyl esters [FAME]) & GCMS analysis

Preparation of Sodium methoxide

About 2.3 g of sodium were measured by analytical balance & transfer to the titration flask. Methanol was added drop wise & mixed well. Finally 100 ml of methanol were added & mixed well.

Method

0.5g of oil sample was measured in to a boiling tube (titration flask) using analytical balance. It was dissolved with 3 ml of benzene & added 5 ml of sodium methoxide, & 10 ml of methanol respectively and mixed well. It was heated on a magnetic string heater for about 1.5 hours at 55oC. Then it was taken from the stir & cool to the room temperature. About 10 ml of distilled water was added to it and mixed well (a white color emulsion will form when the FAME is completely form). After that 10 ml of diethyl ether was added into it

& shaken well, and kept it until the layer separation. The diethyl ether layer was separated by using a dropper & it was concentrated using a water bath. FAME solution was filled into the GCMC vial (Vial was cleaned firstly with distilled water, then diethyl ether & dried in a moisture oven). The prepared vials were run in GCMS machine. Fatty acid profile was carried out to a oil sample which was extracted from fresh wheat germ sample of Australian soft wheat and a oil sample which was extracted from best stabilized wheat germ sample according acid value and statistical analysis of the experiment.

III. RESULTS AND DISCUSSION

Acid value of samples during 6 weeks of storage

Acid value of 8 treatment combinations pertaining to 6 weeks storage (2 week intervals) are given in figure 2

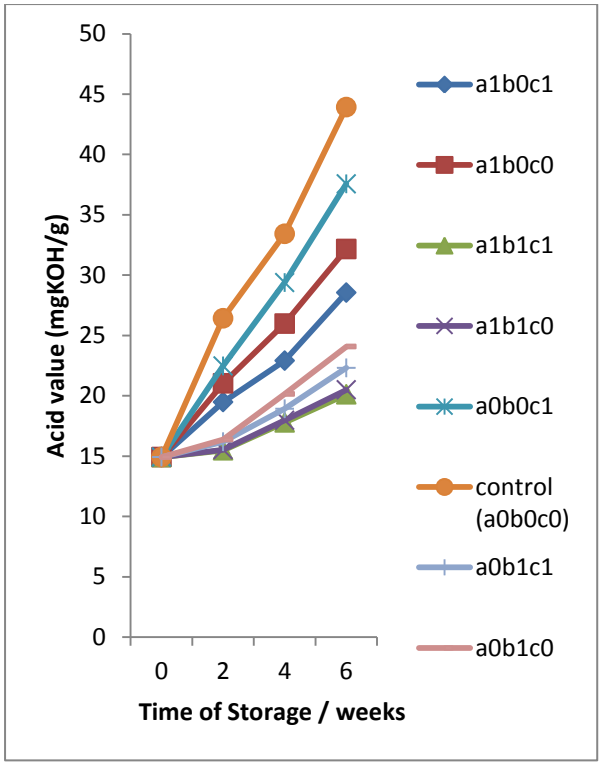

Fig. 2 Development of acid value of treatments during storage

According to figure 2, acid values of all treatments are steadily increasing up to 6 week period of storage except the treatment $a_1b_1c_1$ (Blanched, low moisture and freeze) and $a_1b_1c_0$ (Blanched, low moisture and in-house condition storage) as their increment is least. The bad treatment combination was $a_0b_0c_1$ (un-blanched, moisture uncontrolled and freeze) because, development of acid value of this treatment was as almost same as the control treatment.

Reasons for development of low acid values in best treatments were due to controlling of hydrolytic and oxidative rancidity by lowering moisture content and applying a heat treatment to the wheat germ. Because, rancidity process of oils usually occurs as a result of high moisture content (Hydrolytic rancidity) & presence of enzymes in the host and atmospheric oxygen in the stored environment.

Hydrolytic rancidity is caused by the breaking down of a lipid into its basic component such as fatty acids and glycerol as shown in figure 3.

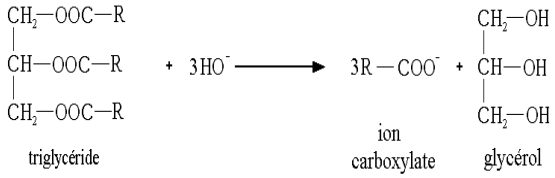

Fig. 3 Hydrolytic rancidity process

Presence of water and lipase enzymes in the wheat germ itself along with high temperature in the stored environment cause to accelerate the rate of hydrolization of fatty acids (Mumtaz shaheen et al, 2010). Developed fatty acids in wheat germ further subjected to auto, photo and enzymatic oxidation process. However, since all of these treatments were kept under in-house and refrigerator conditions influence of photo oxidation is negligible. Moreover treatments undergone with heat were also free from enzymatic oxidation due to destruction of lipases.

Therefore, reason behind the development of low acid value in best treatment was due to destruction of lipase enzymes as a result of the heat treatment. Moreover, as this treatment has undergone moisture reduction (8%), the low moisture content itself retards the hydrolytic rancidity process too. Therefore $a_1b_1c_0$ treatment is the best treatment due to devoid of lipase activity and hydrolytic rancidity. On the other hand, as this treatment was kept under refrigerator condition (-18^{o}C), the low temperature in the storage condition also impedes the rancidity process further.

In order to scrutinize this conclusion further, data were analyzed statistically using ANOVA and F ratio value for each factor.

According to the F table value (F_4^1 = 7.71), the treatments subjected to blanching (a_1), low moisture content (b_1) and storing under refrigerator condition (c_1) having the calculated F ratio value of 39.9, 9.23 and 1.92 respectively.

Statistical analysis also indicate that reducing the moisture content and blanching having a significant effect on stabilizing the wheat germ as F ratio values of them are higher than that of table value. But freezing has not shown any significant influence in preventing the rancidity of wheat germ. Therefore, best treatment in stabilizing the wheat germ is blanching and maintaining it at low moisture content.

Change occurrence on fatty acid profile of wheat germ during storage

To further validate the above results fatty acid profile of fresh wheat germ and best treatment $a_1b_1c_0$ (blanched and low moisture) were analyzed according to GC/MS and results are shown in table 1;

Table 1: Fatty acid profile of fresh and best treated wheat germ oil

IUPAC Name	Fatty acids	percentage of fatty acids in fresh sample	percentage of fatty acids in best sample ($a_1b_1c_0$)
Decanoic acid	Capric acid	0.024	0.029
Dodecanoic acid	Lauric acid	0.031	0.024
Tetradecanoic acid	Myristic acid	0.116	0.104
hexadecanoic acid	Palmitic acid	17.936	17.541
Octadecanoic acid	Stearic acid	0.508	0.496
Octadec-9-enoic acid	Oleic acid	14.324	15.462
9,12-Octadecadienoic acid	Linoleic acid	58.460	56.624
Alpha 9,12,15-octadecatrienoic acid	Linolenic acid	7.964	7.124

The data given in table 1 clearly indicates that fatty acid profile of fresh wheat germ sample is almost similar to the fatty acid profile of best treated sample ($a_1b_1c_0$). Because this treatment more or less devoid of the influence of hydrolytic rancidity and enzymatic rancidity due to low moisture and heat treatment (Inactivation of lipases).

Moreover, saturated and unsaturated fatty acid profiles of fresh wheat germ and best treatment ($a_1b_1c_0$) were also compared and results are shown in figure 4.

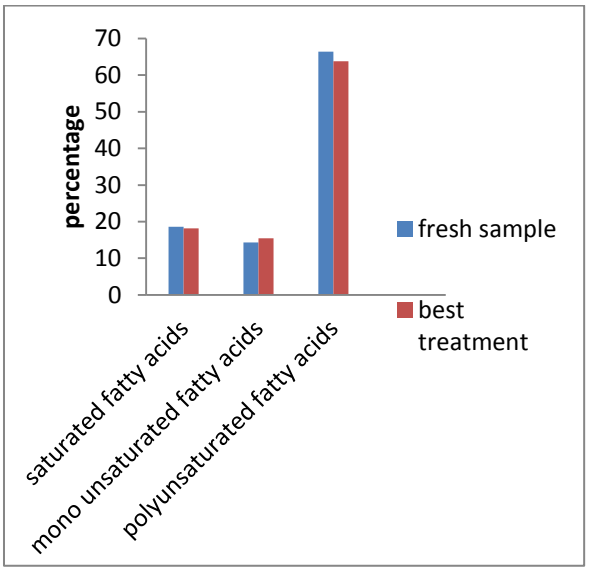

Fig. 4 saturated and unsaturated fatty acid profile of fresh and best treated wheat germ sample

The fig. 4 also clearly illustrates, that best treatments in controlling of acid value of wheat germ is heat treatment and maintaining the low moisture content.

IV. CONCLUSION

Moisture reduction and heat treatment and storing either in refrigerator or in house condition are the best treatments to stabilize the wheat germ specially to control hydrolytic and oxidative rancidity processes. Moisture reduction and heat treatment due to blanching do not affect the fatty acid profile of wheat germ oil. When wheat germ is storing in normal atmospheric condition (RH: 68-72%, Temp: 26-28^{o}C), additional precautions should be taken to prevent the pest attacks. Under this circumstance storing under refrigerator condition is the best. If the wheat germ can be stored under vacuum condition, oxidative rancidity process can also be controlled.

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