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Effect of hydrothermal pre treatment on biochemical properties of red gram

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Abstract: *Pulses are the edible seeds of pod-bearing plants belonging to the family of Leguminosae and are widely grown throughout the world. They are known to be reserves of nutrients providing energy, dietary fibre, protein, minerals and vitamins required for human health. Several research studies suggest that consumption of pulses have several potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol. Parboiling or hydrothermal processing is known to harden the grains and improve the milling yield. This process is commonly applied to rice and other cereals like wheat, barley etc. Nonetheless, the effect of this treatment on the dehulling quality of pulses such as locust bean, pea, pigeon pea, black gram etc., has also been studied by several researchers. The Continuous steaming of red gram was done using a laboratory model of continuous steaming unit. The physiological parameters of protein, fat, fiber and carbohydrate contents for treated samples and untreated sample (control) were analyzed to determine the biochemical composition. There was no significant difference in the observations. There were minor changes observed caused due to the denaturation of protein.*

Key words: *Pulse, Fibre, Protein, Dehulling, Denaturation*

INTRODUCTION

Pulses are the edible seeds of pod-bearing plants belonging to the family of Leguminosae and are widely grown throughout the world. They are known to be reserves of nutrients providing energy, dietary fibre, protein, minerals and vitamins required for human health. Several research studies suggest that consumption of pulses have several potential health benefits including reduced risk of cardiovascular disease, cancer, diabetes, osteoporosis, hypertension, gastrointestinal disorders, adrenal disease and reduction of LDL cholesterol (Hu, 2003; Jacobs & Gallaher, 2004; Philanto & Korhonen, 2003; Tharanathan & Mahadevamma, 2003). Hence pulses are also referred to as "Poor man's meat".

India ranks first in pulse production by contributing about 26% to the global pulse production of 68 million tones tonnes (FAOSTAT, 2011). Red gram (Cajanus canjan L) is one of the most important food legume in tropical and sub-tropical countries (Summerfield and Robberts, 1985) have potential value as an economic source of high protein (Eneche, 1999) well recognized as vegetarian source of Protein. It contains 20–25% protein and is consumed after suitable processing (Tiwari *et al.*, 2008). India is the largest producer of red gram which produces about 64% of total world production. Pulses may be classified as easy-to-dehull and hard-to-dehull based on the ease of seed coat removal. Pigeon pea is most difficult- to-dehull grain among pulses, mainly due to firm attachment of cotyledons by a compound structure of gums and mucilage (Ramakrishnaih & Kurein, 1985) combined with presence of uronic acid in the form of Calcium pectate. Hence, they are usually pre-treated to loosen the hulls before they can be separated by mechanical means to increase the recovery of dhal (Sahay *et al.*, 1985; Mangaraj *et al.*, 2004). Dehulling is defined as the removal of the outer hull (fibrous seed coat or testa) which is tightly attached to the cotyledons, usually via a thin layer of gums and mucilages along with uronic acids in the form of calcium pectate (Kurien & Ramakrishnaiah, 1985). Pre-treatment is required prior to removal of the hull to: (a) loosen the hull, (b) ease milling, (c) reduce breakage and (d) improve the quality of splits (Tiwari, Jagan Mohan, & Vasan, 2007).

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Parboiling or hydrothermal processing is known to harden the grains and improve the milling yield. This process is commonly applied to rice and other cereals like wheat, barley etc. Nonetheless, the effect of this treatment on the dehulling quality of pulses such as locust bean, pea, pigeon pea, black gram etc., has also been studied by several researchers (Adewumi & Igbeka, 1993; Urbano *et al.,* 2003; Opoku *et al.*, 2003; Tiwari *et al.*, 2008a; Tiwari *et al.*, 2010). The term parboiling has been adapted in the current work to describe the operations carried out viz., moisture conditioning, steaming and drying. Parboiling was observed to increase the milling yield, dehulling efficiency and reduce the powder losses (Tiwari *et al.*, 2010).

MATERIALS

Grain Sample

The sample used for this study was red gram of TS-3R wilt resistant variety. This was procured from Gulbarga market, Karnataka. The sample was checked for moisture content and this was determined as suggested by Henderson *et al*. (1997). Ten gram of red gram sample was taken in triplicates placed in pre-measured moisture boxes. The moisture boxes were placed in a hot air oven (Everflow oven, Chennai, India) and set at 130°C for 18 h (ASAE, 2003). This method was also confirmed using a digital moisture meter (Model: Indosaw, Make: Osaw industrial products pvt. Ltd., Haryana, India). The first grade red gram is graded with size more than 3 mm. Grade 1 red gram was used for experimental study.

Continuous Steaming Unit

The Continuous steaming of red gram was done using a laboratory model of continuous steaming unit designed and developed at IICPT. This model was designed for paddy and millet processing (Fig. 1). This unit consists of a horizontal auger mechanism that uses a rotating helical screw within a U-shaped trough, to move the grain. The diameter of the screw is 150 mm with a pitch of 150 mm. The length of the screw was 920 mm. The screw was mounted on a hollow shaft of 33 mm diameter. The top of the trough is tightly closed. The shaft of the screw is driven by a motor whose speed was reduced by a reduction gear box (40:1) with chain and sprockets. A variable speed drive was used to vary the rpm of motor in order to vary the residence time of the grain in the steaming chamber. The grain temperature was measured using thermometer, which is immersed in the grains. Steam was generated in a diesel fired boiler of 100kg/h capacity and supplied continuously into the chamber of screw conveyor in a parallel flow pattern through galvanized iron pipe of 15 mm diameter. The inlet steam pressure was monitored using a pressure gauge and adjusted by a valve.

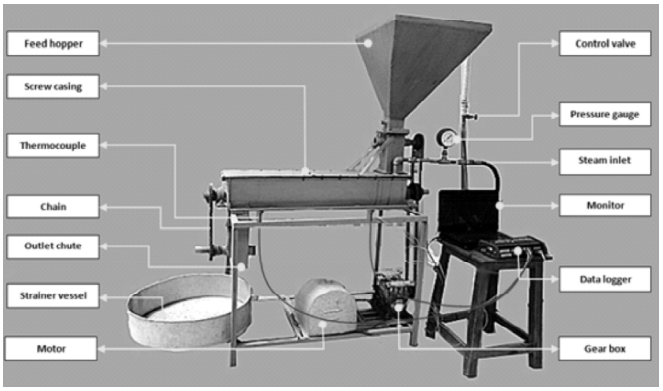

Figure 1: Continuous steaming unit

Experimental Design

The effect of hydrothermal process variables on the dehulling qualities and final product quality characteristics of red gram was studied using three levels of initial moisture content (10%, 14% & 18% wet basis (w.b.)), three levels of inlet steam pressure (2, 3 & 4 kg/cm^2) and three levels of grain residence time (2, 4 & 6 min). The continuous steaming unit was selected for the evaluation. Preliminary studies were conducted and the levels of the initial moisture content, inlet steam pressure and grain residence time were selected. A 3^3 full factorial design was employed for the experiment. 3 factors were IMC, ISP and GRT. The design included 27 experiments with 3 replications for each experiment. The detailed flowchart of the experiment conducted is given in Fig. 2.

PROCEDURE

Moisture Conditioning

Moisture Conditioning to Higher Moisture Content

The grains were checked for initial moisture content determined by oven method (ASAE, 2003) and it was 11%. Water was added for conditioning the grains to the desired moisture content (14% & 18% w.b.), which was calculated using Eq.1 [AACC Method 26-95 (AACC, 1995)]. The calculated amount of water was added to the grains by spraying.

$$W_W = \left(\frac{100 - Mo}{100 - Md} - 1\right) \times Ws \quad (1)$$

Where W_w is the weight of water to add (g)

W_s is the weight of the grain sample (g)

M_o is the original moisture content of the grain sample (% w.b.) and

M_d is the desired moisture content of the grain sample (% w.b.).

The conditioned samples were tempered carefully by mixing in a box manually for even absorption of water added. The conditioned samples were then packed into separate polyethylene bags. The bags were sealed tightly and the samples were kept at 5°C (±1°C) in a cold storage chamber for 7 days. This was done for the moisture to distribute uniformly throughout the samples (Carman, 1996; Aydin *et al*., 2002). The final moisture content of the samples were determined and samples were stored for experiments.

Prior to running of experiment, the sample was taken out of the storage environment and placed at the ambient environment until its temperature was close to ambient air temperature for about 2 h (Yalcin & Ozarslan, 2004; Isik, 2007).

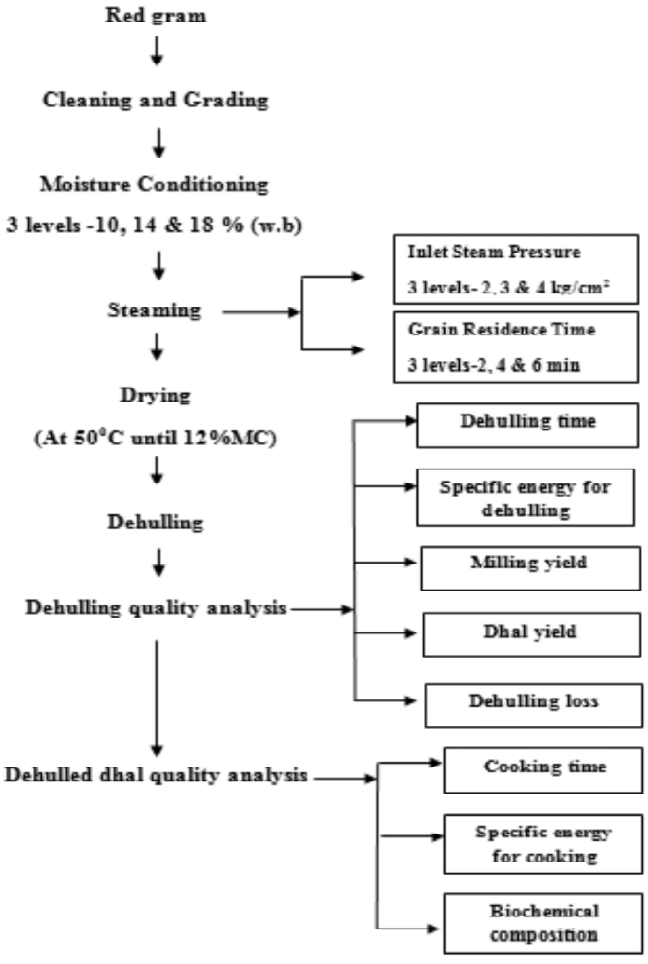

Figure 2: Flow chat of experiment conducted

Steaming

The continuous steaming unit as described in previous section was used for steaming of red gram sample. About 3 kg of red gram sample was taken and loaded in the hopper of the continuous steaming unit. Steam flow was set by adjusting a control valve to particular inlet pressure level, for saturated steam generation. This was allowed to stabilize for few minutes. Three steam inlet pressures of 2, 3 and 4 kg/cm^2. The desired grain residence time was set adjusting by setting rpm of motor using a variable speed drive. GRT of 2, 4 and 6 min was used in this study.

The ISP was stabilized and indicated by observing indicator (pressure gauge) after which the feed inlet shutter attached to the hopper was opened fully and the grains were allowed to flow in to the steaming chamber. A strainer vessel was placed at the outlet chute of the unit to remove condense water from the grain surfaces. The procedure was repeated for all inlet steam pressures and grain residence times. A mercury was thermometer inserted at the top of the screw conveyor, for measuring the grain temperature during steaming. The grain temperature was monitored similarly by placing thermometer inside the grain.

Drying

Drying of the steamed grains was done by spreading on stainless steel trays and placing under a shade for 1h. This reduced the grain temperature to ambient air temperature and dried the surface moisture of the grain. The trays with semidried steamed grains were placed in an electric tray drier (Industrial and laboratory tools corporation, Chennai, India) set at 50°C for obtaining a final moisture of 12% (w.b.). The 12% moisture content is considered the desired moisture content for dehulling pulses (Sreenarayanan & Devadas, 2000). Tray dried grains were checked for moisture content reading every 1hr during drying. The grains were stirred and the grain positions rotated. Moisture of the grains were determined within 2 min of their removal from the drier as recommended by Bhattacharya & Swamy (1967) using a digital moisture meter (Model: Indosaw, Make: Osaw industrial products pvt. Ltd., Haryana, India) which was calibrated under the identical conditions of operation against the ASAE (2003) oven method.

The drying was stopped once the desired moisture content of 12% (w.b.) reached. The samples were removed from the tray drier and required quantity of samples were taken and packed in air-tight polyethylene bags. The packed samples were stored at room temperature for further analysis.

Conventional Oil Pretreatment

For pitting of seed coat of 200g red gram grains weighing were taken and passed once into a laboratory model emery roll polisher which caused 75–80%. The pitted grains were treated with 0.5% peanut oil and dried under sun for 2 to 3 days. They were sprayed with 2.5% water and the grains were heaped overnight for tempering. Then the grains were dehulled (Kurien, 1987).

Control sample were the sample had no oil pre treatment or hydrothermal treatment.

Dehulling Procedure

A laboratory model emery roll polisher (Model: TM05, Satake Corporation, Japan) was used for dehulling of red gram was done till the husk/hull of red gram was completely removed for all grains and corresponding time was noted. After dehulling, fractions of milled grains using sieve analysis were collected and graded according to as dehulled whole, split, husk, broken, powder and fine brokens. They weighed separately digital weighing balance. Samples were packed for further analysis stored in air-tight polyethylene bags (Fig. 3). The procedure for all samples was done in triplicates.

Figure 3: Red gram dhal samples packed in air-tight polyethylene bags

Effect of hydrothermal treatment on biochemical composition of red gram

These analysis were carried out to test whether there is any losses of Biochemical composition like Protein, Fibre, Fat and Carbohydrates in Hydrothermally treated sample compared with controlled sample.

Protein Analysis

Two g of samples were (Parboiled and controlled sample) taken in digestion tubes. 5 g of Potassium sulphate and 1 g of Copper sulphate was added, 10 ml conc. Sulfuric acid into tubes was added. The tubes were placed in Digestion chamber, digestion is carried out until the solution turns green colour. Sample is removed from digestion block and place the tubes still cover onto stand and run scrubber till all acid flumes are gone. 40% NaOH in in the alkali tank of distillation unit is placed. The digestion flask was connected to distillation bulb on condenser and with the tip of the condenser immersed in a standard 4% boric acid solution. Collect the steam of the distillate after the distillation completed. Remove the receiving flask. Titrate boric acid receiving solution with standard 0.1 N HCl add 2-3 drops of mixed indicator in conical flask and titrate light pink appears.

Determination of Protein is done with following equations (2) and (3)

$$\%N = \frac{14 \times normality\ of\ acid \times (titrate\ value - 0.2)}{Sample\ weight \times 10} \quad (2)$$

$$\text{Crude Protein} = \frac{\%N}{F} \ldots\ldots\ldots \quad (3)$$

F - 6.25 for pulses

Crude Fiber Estimation

Two g of sample was taken (Parboiled and Controlled sample) in a beaker, 1.25 ml of H_2SO_4 solution is added and made upto 100ml with distil water. Heated on hot plate for half an hour. The solution is filtered with Muslin cloth, the residue is taken in beaker and 1.25 g of NaOH is added and made upto 100 ml wit distil water. Again heated on hot plate for half an hour. Filter the solution in Muslin cloth. The residue is collected and kept for drying on known empty weight of filter paper. Dried it for 2.5 hrs (110-130 °C). The weight is taken after drying.

The amount of fibre is calculated by following equation (4)

$$Fibre = \frac{Final\ weight - Empty\ weight}{Sample\ weight} \times 100 \quad (4)$$

Estimation of Fat

One to 5 g of dehulled grains (Parboiled and Controlled sample) are taken directly into tared cellulose timbles. Dry the timbles containing sample at 102±2^{0}C for 2 hrs. Place the defatted cotton on top of the timble covering the sample. All three, i.e., solvent, samples and cotton must be free of moisture to avoid extraction of water-soluble components such as carbohydrates, urea, lactic acid and glycerol which will result in false high values. Turn on Soxtherm fat extractor and condenser cooling water. Put 80 ml of solvent (hexane) into each extraction flask to cover test portion when timbles are in boiling position. Place flask under extraction columns and secure in place. Lower thimbles into solvent and boil for 6 h. Raise thimbles out of solvent and extract in this position for 40 mins. Then distill as much as possible from flask to reclaim solvent and attain apparent dryness. Remove extraction flask from extractor and place in operating fume hood to finish evaporating solvent at low temperature. Dry extraction flask in 102±2 °C oven for 30 mins to remove moisture. Excessive drying may oxidize fat and give high results. Cool in condenser to room temperature and weigh to nearest 0.1mg.

This Fat can be determined by following equation (5)

$$Fat\% = \frac{w_2 - w_0}{w_1} \times 100 \quad (5)$$

Where,

W_0 – Empty flask weight

W_1 – Sample weight

W_2 – Flask weight + Fat residue

Carbohydrates Estimation

Sample of 0.1 g is taken (Control and Parboiled sample) in beaker, 5 ml of 2.5 N HCl is taken. Heated it at 70 °C for 3 h. Cool the sample and add $NaCO_3$ till effervesenses stops. The sample is filtered with help of Wattman filter paper, the filterate is made upto 100ml with distilled water. Then the sample is taken in 3 test tubes at different quantity 0.4, 0.6 and 0.8, then it made upto 1 mL by distill water. 4 ml of anthrone solution is added to each tube, heated it for 8 min then cooled. Then read at 630 nm in Spectrophotometer by keeping anthrone as blank. Carbohydrate is calculated by the following equation……..6

Carbohydrate = 20*(Sample wavelength/ standard wave length) (6)

Standard Wavelength = 0.193

Effect of Hydrothermal treatment on biochemical compositions of red gram

The physiological parameters of protein, fat, fiber and carbohydrate contents for treated samples and untreated sample (control) (Table 1) were analyzed to determine the biochemical composition.

There was no significant difference in the observations as shown in Table 2. There were minor changes observed caused due to the denaturation of protein. Similar study was conducted by Tiwari *et al.*, (2008) which reported no significant difference in terms of crude protein and carbohydrates content for hydrothermal pre treatment and untreated sample for batch process. The results obtained in the present study are in conformity with their result.

Table 1
Biochemical parameters of control and hydrothermal treated sample

Sample	*Fat*	*Fibre*	*Protein*	*Carbohydrate*
Control	1.3±0.020	1.0	25.73±0.07	53.55±0.16
Hydrothermal treated	1.2±0.017	1.0	24.45±0.15	52.2±0.26

Table 2
Paired comparison test for the biochemical parameters for two different types of treatments

	Control	*Hydro thermal treated*
Mean	20.4525	19.855
Variance	626.439025	599.4867667
Observations	4	4
Pearson Correlation	0.999913204	
Hypothesized Mean Difference	0	
Df	3	
t Stat	1.883135559	
P(T<=t) one-tail	0.078105058 ^{ns}	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.156210116 ^{ns}	
t Critical two-tail	3.182446305	

ns- not significant

CONCLUSION

The study showed that continuous hydrothermally treatment caused a little loss in terms of protein, fat, fibre and carbohydrates. However the reduction was insignificant at 5% level of significance.

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