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Studies on influence of different temperatures on the respiration rate of soybean (*Glycine max* L., Merrill) seeds

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Abstract

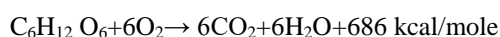
Soybean is an important protein rich oilseed crop that is cultivated in the tropical, sub-tropical and temperate regions. It is gaining worldwide importance due to its high nutritional benefits. As soybean seeds are oleaginous, they are easily prone to oxidative damage apart from this exposure of soybean to high temperatures in the tropics makes storage for longer period difficult. Analysing respiration rate at different temperatures is important to study the effect of storage temperatures on extending the viability and quality parameters of the soybean seed. Measurement of Respiration rates was carried out by conducting experiments at three different temperatures, 30 °C, 15 °C and 5 °C. The respiration rates were calculated as the rate of production of CO₂ equivalent to the rate of O₂ consumption of the soybean seeds. The CO₂ production and O₂ consumption is maximum at a higher temperature of 30 °C and minimum at 5 °C. The respiration rate gradually decreases at 5 °C which correlates to a decline in respiration when the temperature is low.

Keywords: Soybean, seed respiration rate, temperature, time, O₂ and CO₂ concentrations

1. Introduction

Soybean (*Glycine max* L., Merrill) is an important protein rich oilseed crop (Anderson *et al.*, 2019) belonging to the family Fabaceae and is widely grown in tropical, subtropical as well as temperate regions and has its origin in East Asia. In the global scenario, in the year 2020-2021 Brazil tops the list in global soybean production with 144 million metric tonnes, followed by the United States of America with 119.88 million metric tonnes and Argentina ranks third in the list. India ranks fifth among the countries in the world with around 11.2 million metric tonnes of production capacity (Oilseeds - World Markets and Trade, USDA Publication). Demand for high-quality plant-based protein will continue to increase the need for soybean meal, primarily for the animal feed and human food industries over the period forecasted. The main reasons behind the increasing demand for soybean is the health benefits associated with this protein-enriched food and the good fat content. The commercial exploitation of soybean in India is five decades old and according to the Directorate of oilseeds development, the 2020 *Kharif* season soybean cultivation took place on an area of 121.927 lakh hectares of land, the production estimated to 112.259 lakh tonnes and the yield was calculated to 105 lakh tonnes and productivity being 921 kg/ha with Madhya Pradesh as the leading state in production. Soybean seeds contain about 35-45 % protein, 15-25 % of oil, 33% carbohydrates of which 16.6% are soluble sugars, 5% crude fibre, and 5% of ash (Hou *et al.* 2009). Soybean seeds are oleaginous due to high oil content and are prone to rapid deterioration in quality, starting from the field right after physiological maturity till storage. In the soybean oil fraction, linoleic acid represents about 54%, while linolenic acid accounts for 7 to 9 %. These two fatty acids stand out as the most susceptible to enzymatic and non-enzymatic oxidative deterioration which in turn deteriorates the seed quality (Dijkstra, 2016) [3]. Oxidative mechanisms usually increase respiration and consumption of ATP in the cells of seeds (Tiwari *et al.*, 2002) [15]. Also, soybean being structurally fragile, micro-biotic, short lived and easily subject to mechanical damage, storing them is a challenge to the seed industry as it loses viability and germination percent below the standards of minimum seed certification before the next growing season. The seed being a biological material, lose their freshness, vigour and viability due to uncontrolled physiological and biochemical activities that take place when stored under ambient conditions. Respiration is a physiologically unavoidable catabolic process and is highly energy demanding where complex organic material is continuously broken down into

simpler forms by utilizing O₂ and evolving CO₂, H₂O and energy required for the living tissue (Goswami *et al.*, 2010)^[7] and other molecules which can be used by the cell for synthetic reactions (Wills *et al.*, 1989)^[17]. Respiration is necessary to keep the metabolic process functioning efficiently, but it also hastens loss in viability, which is not ideal for extending shelf life. Respiration results in the loss of moisture from the fresh produce which results in shrinkage and physiological loss in weight. It takes place not only due to the cellular respiration process but also in the gas exchange process (Andrich *et al.*, 1998)^[2]. The extent of respiration can be measured by determining the amount of substrate loss, oxygen consumed, carbon dioxide liberated, the heat produced and energy evolved. The metabolic reaction during respiration is as below:



All forms of respiration in the presence of oxygen results in the biochemical conversion of stored food substrates, such as glucose into energy. Respiring tissue consumes oxygen, and the amount lost can be used to quantify the rate of aerobic respiration. The respiration rate of the living tissues can be decreased as a result of the decreased oxygen and increased carbon dioxide in its surrounding atmosphere or by reducing the storage temperature. Less respiration rate and longer viability result from this decreased energy available for chemical changes. By observing how quickly the substrates break down and regenerate into new ones, the rate of any reaction can be calculated. Since carbon dioxide in the atmospheric air has a concentration of about 0.03 percent, changes in production that are even slightly different would be easy to notice because they would represent a significant increase over the background concentration. Therefore, the preferred method used to estimate respiration is by measuring the carbon dioxide production rates (Kader and Saltveit, 2002)^[9]. Calculating respiration rates under different temperatures and storing seeds at optimum conditions play an important role in maintaining seed viability and quality. Usually, a biological entity with higher rates of respiration tends to have shorter storage life than those with lower rates of respiration.

To maintain the quality and to extend its viability during storage, respiration rates have to be minimized. Hence soybean seeds were stored at different temperatures to study the respiration rates.

2. Materials and methods

2.1 Materials

Soybean seeds variety DSb 34 were purchased from UAS, Dharwad and the initial moisture was measured as 10 %. For the studies on the respiration rate of the soybean seed sample, experimental storage chambers consisted of glass bottles of 1000 ml capacity with an airtight lid. Two holes were made on the lid and the septum was attached to a bolt and nut type screw and was fixed into the holes. After the samples were placed inside, the mouth of the bottle was wrapped with teflon tape to provide air tight conditions.

2.2 Experimental methods

The respiration rate was calculated as suggested by Kader and Saltveit (2002)^[9] and Susana *et al.* (2002).

$$R_{O_2} = \frac{\Delta O_2 \times V}{M \times T} \text{----- (1)}$$

$$R_{CO_2} = \frac{\Delta CO_2 \times V}{M \times T} \text{----- (2)}$$

Where,

RO ₂ and RCO ₂	- 3	Respiration rate in terms of O ₂ consumed and CO ₂ evolved respectively (ml kg ⁻¹ h ⁻¹)
V	- 4	Free volume inside glass bottle (ml)
Δ O ₂	- 5	Difference in the volumetric concentration of O ₂ at initial and final time respectively (%)
Δ CO ₂	- 6	Difference in the volumetric concentration of CO ₂ at initial and final time respectively (%)
M	- 7	Mass of the stored product (kg)
T	- 8	Difference between the initial and final time (hour)

The respiration rates of soybean seeds were noted at three different temperatures of 30°C, 15°C and 5°C. Glass bottles with 1000 ml capacity were taken and 250 g of soybean seeds were weighed and placed inside the glass bottles. Teflon tapes were wrapped around the lid to provide gas-tight conditions. The lid contains the airtight silicon rubber septum through which the gas inside the bottle can be sampled using a needle attached to the gas analyser without any leakage. Three such replicates for each of the temperatures *viz.*, 30°C, 15°C and 5°C were maintained. Soybean seeds were allowed to respire inside the glass bottles placed in the experimental storage chambers. The gas concentrations inside the chambers were measured using O₂ - CO₂ Gas Analyzer (Make: PBI Dan sensor, UK; Model: Checkmate II). The gas samples were drawn from zeroth hour and at every one-hour interval. When the syringe is introduced into the rubber septum attached to the lid of the glass bottle, the inbuilt pump system automatically samples the gas through the needle at the end of the system, ensuring an accurate operation and calculation. The gas analysis results were shown in the built-in LCD display and also stored in the memory. The results can then be exported to an external computer via the USB data connection or saved on a memory stick or printed on the built-in printer. The average length, width, thickness, bulk density and the 100 seed weight of soybean seeds were also analysed.

3. Results and Discussion

3.1 Physical properties

The average length, width and thickness of the soybean seeds were 3.21mm, 1.95mm and 0.68mm respectively. The average bulk density and the 100 seed weight of soybean seeds were 674.30 kg/ m³ and 8.948 g respectively.

3.2 Changes in the concentration of gases with respect to temperature and time

The metabolic reactions due to respiration show variation in the gas concentration of soybean seeds stored in the airtight glass bottles under all three temperatures. With progress of time, there was a decrease in the concentration of O₂ and an increase in the concentration of CO₂. The changes in the concentrations of O₂ and CO₂ at 30°C, 15°C and 5°C with an increase in time are represented in Figure. 1. and Figure. 2.

3.3 O₂ consumption rate

The O₂ consumption rate decreased significantly in the airtight glass bottles, with a decrease in storage temperature. Also, as time increased from 0 to 53 hours, there was a gradual reduction in the O₂ concentration irrespective of the storage temperatures. The consumption of O₂ was minimal in soybean seeds stored at 5°C than at 15°C and 30°C.

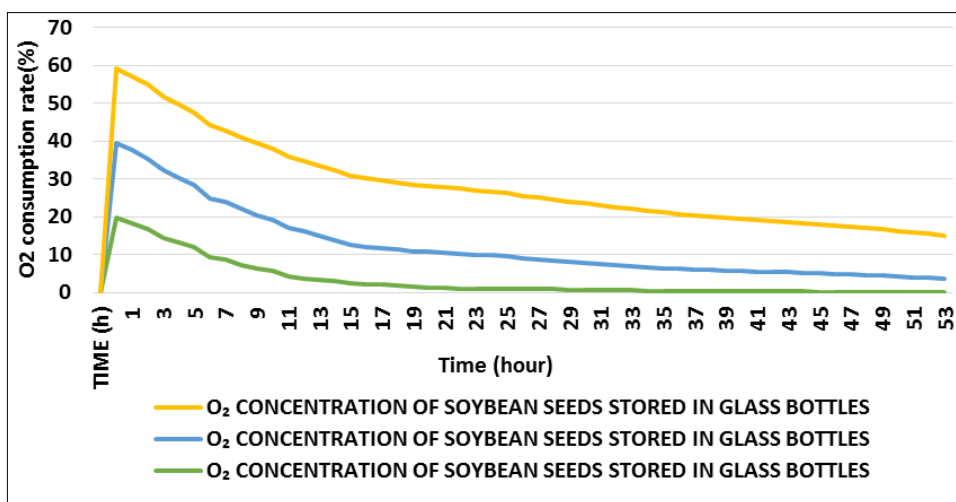


Fig 1: Influence of O2 concentrations with respect to time at different temperatures on soybean seeds

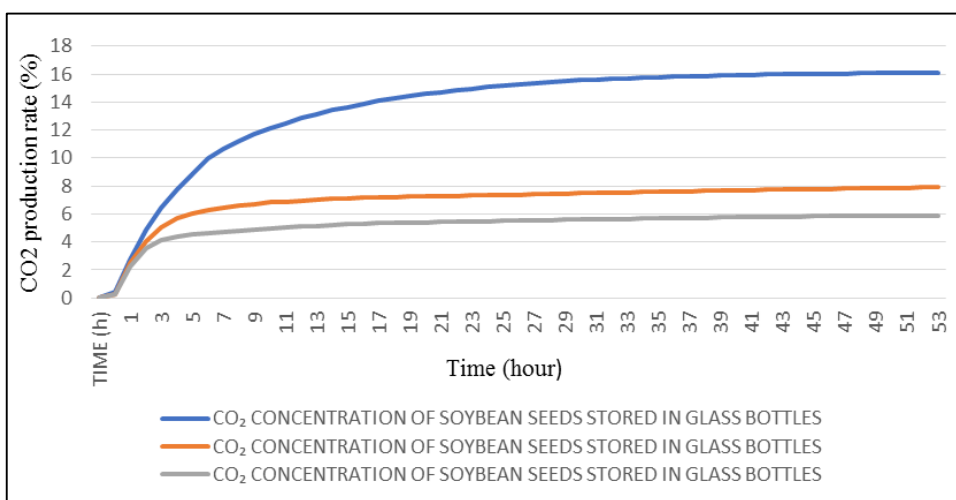


Fig 2: Influence of CO2 concentration with respect to time at different temperatures on soybean seeds

3.4 CO₂ production rate

At the same time production of CO₂ increased steadily with time and an increase in concentration was independent of the storage temperature. The CO₂ production rate increased significantly as the temperature increased. The CO₂ production rate was elevated in the soybean seeds stored in airtight glass bottles at 30°C compared to the soybean seeds stored at 15°C and 5°C. Among the storage temperatures, soybean seeds stored at 5°C produced CO₂ at lower levels.

Yang *et al.* (1988) stated that during respiration the loss of O₂ and the gain in CO₂ concentration inside the closed container will spoil the product quality due to the production of internal

heat from the seeds. According to Lakakul *et al.* (1999), the rise in CO₂ concentration in the package or a jar will increase the heat where there will be a chance of enhancing the rate of respiration with increase in time. In a study on respiration rate of field bean at different temperatures by Nisha *et al.* (2017), the rate of CO₂ production was higher at 24°C in comparison to 10°C and 3°C. In parallel to the CO₂ production, a decrease in respiration rates was observed at 10°C and 3°C. Similar results were observed in this experiment where an increase in temperature increases the respiration rate which is represented in Table. 1. and Figure. 3. The respiration rate was calculated using equation (2).

Table 1: Respiration Rates of soybean seeds at 1 hour time interval

Time (hours)	R CO ₂ at 30°C	R CO ₂ at 15°C	R CO ₂ at 5°C
1	92.40	60.12	32.92
2	87.20	41.20	19.56
3	63.20	24.48	12.48
4	50.80	17.88	8.24
5	44.80	13.64	5.80
6	44.40	10.24	3.92
7	26.80	8.12	3.40
8	24.00	6.16	3.28
9	20.00	4.36	3.16
10	15.60	3.64	2.96

11	14.80	3.00	2.84
12	13.60	2.56	2.60
13	12.40	2.36	2.36
14	10.40	2.04	2.08
15	9.60	1.84	1.84
16	8.80	1.56	1.52
17	8.40	1.12	1.24
18	7.60	1.00	1.08
19	6.40	0.96	0.96
20	6.00	0.95	0.88
21	5.20	0.93	0.82
22	5.20	0.92	0.83
23	4.80	0.90	0.82
24	4.80	0.89	0.80
25	4.00	0.88	0.79
26	3.60	0.87	0.78
27	3.20	0.87	0.77
28	3.20	0.87	0.76
29	2.80	0.86	0.75
30	2.40	0.86	0.72
31	2.00	0.86	0.70
32	2.00	0.86	0.69
33	2.00	0.86	0.67
34	1.60	0.85	0.66
35	1.60	0.85	0.65
36	1.20	0.85	0.64
37	1.20	0.84	0.63
38	1.08	0.84	0.62
39	1.00	0.84	0.61
40	0.88	0.82	0.60
41	0.80	0.82	0.56
42	0.76	0.80	0.54
43	0.68	0.77	0.51
44	0.64	0.74	0.48
45	0.62	0.71	0.46
46	0.60	0.68	0.43
47	0.58	0.67	0.40
48	0.56	0.65	0.28
49	0.54	0.62	0.24
50	0.52	0.58	0.22
51	0.50	0.55	0.20
52	0.48	0.50	0.20
53	0.44	0.43	0.19

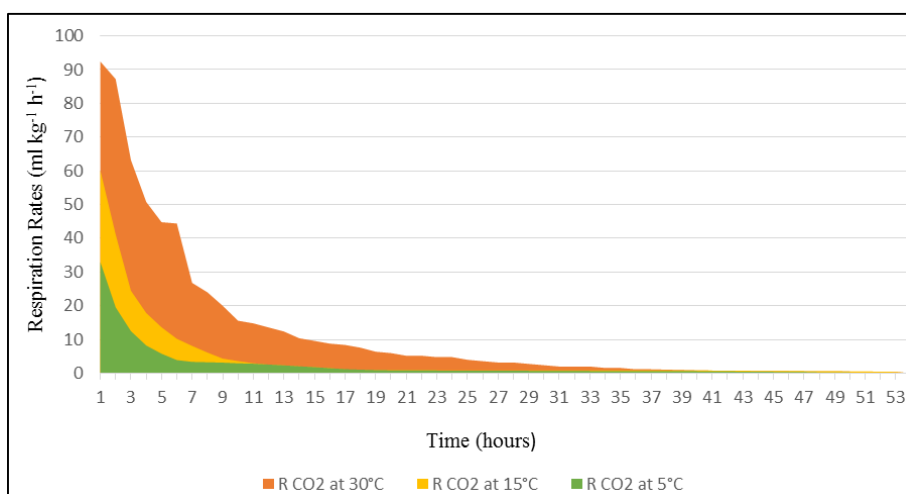


Fig 3: Respiration rate of soybean seeds in terms of CO₂ concentrations

4. Conclusion

In summary it is concluded that the CO₂ production and O₂ consumption rates were faster during the initial hours of

storage and this was irrespective of the storage temperature. But it was clear that at 30°C the respiration rate was the highest in the initial hours and it decreased gradually after 20

hours of storage; whereas at 15 °C and by and large at 5 °C, the respiration rate decreased gradually from the start. This infers that storing soybean seeds at low temperatures would decrease the respiration rate, slowing down the catabolic respiration process in the soybean seeds. The low temperature drops heat production to negligible amounts causing the seeds to be viable for longer time periods.

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