International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2023; 11(4): 35-38

Received: 12-06-2023
Accepted: 19-07-2023

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Microbiological evaluation and shelf-life extension of strawberries dried under infrared-green hybrid drying (IRGHD) Method

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Abstract
Fruits are usually more susceptible to spoilage due to their high moisture content and presence of sugars causing a decreased shelf-life storability, less shelf life and food losses. Strawberry (Fragaria x ananassa) possesses a high nutrition content due to high levels of antioxidants. To preserve the quality and extend the shelf-life of strawberries, a drying method called Infrared-Green Hybrid Drying (IRGHD) was used in comparison with passive and conventional sun drying. The samples were dried from all three means and a comparative study was performed from zero to ninety days' time. Results showed that the IRGHD-dried strawberries were microbiologically sound, with lower bacteria, yeast and mould and coliform as compared to passive and sun-dried strawberries. The antioxidant properties, including total phenolic content, 2, 2-diphenyl-1-picrylhydrazyl, and total flavonoid content, were within the acceptable range and were well preserved after drying. The dried strawberries obtained through this method therefore can be preserved for longer than 90 days with very less or no changes in the antioxidant content.

Keywords: Strawberries, spoilage, high moisture content, sugars, shelf-life, storability, food losses, drying method, Infrared-Green Hybrid Drying (IRGHD), comparative study, microbiologically sound, bacteria, yeast, mould, coliform, antioxidant properties, total phenolic content

Introduction
Strawberries (Fragaria ananassa) are one of the most consumed fruits due to its sensory qualities (taste, colour), nutritional content, and popularity. It is a non-climateric fruit that is frequently consumed year-round throughout the world. Strawberries are indigenous to temperate climates all around the planet. In order to reach markets outside of the growing season, it is cultivated in greenhouses, soilless media, or annual or perennial production systems in the field. In 2019, there were 8,885.028 tonnes of strawberries produced worldwide. USA, Turkey, Spain, Egypt, and Mexico are the top producers of strawberries. Strawberry is grown in the highlands of India. Nanital and Dehradun in Uttar Pradesh, Maharashtra, Kashmir Valley, Bangalore, and West Bengal are its production zones. Fresh market strawberries make up around 83% of the overall production, while the remaining 15% is used for industrial processing, such as the creation of yoghurt, jams, jellies, dessert toppings, etc. Strawberry production has nearly doubled over the past 15 years. Reducing Cardiovascular diseases, controlling blood pressure, anti-cancer and anti-inflammatory properties, as well as a host of other health benefits, are just a few of the advantages that strawberries exhibit. The high concentration of phenolic compounds, vitamin C, anthocyanins, proanthocyanidins (derivatives of cyanidin and pelargonidin), and other antioxidants, which counteract oxidative stress and slow cellular ageing, are responsible for these effects. Due to their naturally low pH, berries are often regarded as low-risk foods. Because strawberries have a very high moisture content, it is difficult to keep the product safe from microbial contamination. It is exceedingly challenging to commercialise because of its high susceptibility to microbial degradation. It can be eaten fresh or in a variety of other forms, including juice, concentrated jam, jelly, dried and rehydrated baked goods, jam, and jelly. The flavour and appearance of a fresh strawberry are influenced by the temperature and relative humidity of the ambient air. Therefore, it is important to consider food preservation methods that can extend food shelf life while preserving vitamins, minerals, and volatile and phytochemical substances.

Drying strawberries allows for the potential to increase their shelf life and create new goods, making it one of the most crucial techniques. It is a tried-and-true method of preserving food where the conditions of the procedure are under your control.

In order to prevent the growth of microbes and extend the shelf life of the product, the primary goal is to remove water until the water activity is low enough. The drying procedure, however, alters the product's shape, crispness, hardness, flavour, and nutritional value in addition to reducing the water content of the product. All elements that affect the fruit's nature, such as phenol content, soluble fibre content, and vitamins, must be taken into account when drying strawberries. To minimise unfavourable discoloration in the finished product, it must be shielded from direct solar exposure. In the same drying conditions, infrared heating has a number of benefits over conventional drying. The infrared radiation method is faster than convection methods, according to studies contrasting it with methods based on air convection. Due to its advantages for enhancing drying efficiency, infrared radiation (IR) has been used in conjunction with a number of drying procedures.

The production of electricity and solar thermal applications employ solar energy, which is a significant renewable energy source. For drying agricultural products, solar air collectors are frequently utilised. Systems that use solar assistance for drying may be used independently or in conjunction with other drying systems. The primary benefits of employing such hybrid drying systems are time savings and increased drying effectiveness.

Materials and Methods

Procurement of raw material

Fresh matured strawberry fruits were purchased at random from a local market in Gauss Hazratbal, Srinagar. Individual strawberries had their stalks and sepals removed and were cut in half. The sample were dried using different drying methods (sun drying, passive infrared assisted green hybrid drying, and active infrared assisted green hybrid drying). After drying the samples were sealed in zip lock pouches and well labelled for further analysis.

Microbiology

All the statistical analysis were carried out using ANOVA

a) Procedure for total plate count

All glassware were sterilized in an autoclave for 10 to 20 mins at 121 °C. The media agar was prepared by weighing 7g and was dissolved in 250 ml of water. The serial dilution of the four samples was dole out by pipetting 1ml of every sample to already measured 9 ml diluted water into a tube labelled 10^{-1}-10^{-4} and was covered with non-absorbent cotton wool to avoid contamination. From the acceptable dilutions (10^{-1}-10^{-4}) 1ml or 0.1ml of suspension was transferred while in motion, with the respective pipettes, to sterile petri dishes. Approximately 20ml of the nutrient medium, melted and cooled to 45 °C, was added to every Petri plate containing the diluted sample. The contents of every plate were mixed by rotating gently to distribute the cells throughout the medium. The plates were allowed to solidify and incubate in an inverted position for 24-48 hours at 37 °C.

b) Procedure for yeast and mould count

In this method, a specific volume, usually 1 mL, of the serially diluted liquid sample was properly mixed with approximately 15 mL of potato dextrose agar in a petri plate. The medium was allowed to solidify before being incubated for 24 to 48 hours at 37 °C. Following incubation, the viable microorganisms in the sample form visible colonies on the surface and within the medium. The visible colonies were counted, and CFU/mL was calculated by using the formula below;

$$\mathrm{CFU/mL} = \frac{\text{Total number of colonies obtained} \times \text{dilution factor}}{\text{volume of specimen used (aliquot)}}$$

c) Procedure for total coliform microorganisms

To count total coliform microorganisms (TC), 1 mL of dilution was transferred to sterile Petri dishes in duplicate, and 12 -15 mL of VRBL (Violet Red Bile Lactose Agar, Biokar Diagnostics) was poured into each Petri dish and carefully mixed with the inoculum. After allowing the mixture to solidify, 4 - 6 mL of the VRBL medium was poured onto the surface of the inoculated medium and allowed to solidify before incubating at 37±1 °C for 24±2 hours as defined in ISO 4832:2006. After 24 hours of incubation, violet colonies with a diameter of 0.5 mm were counted (sometimes colonies were red covered).

Antioxidant analysis

a) Determination of Total Phenolic Content (TPC)

The total phenolic content of the dried strawberries will be determined using the Folin-Ciocalteu colorimetric method. 1gram sample will be dissolved in 70% methanol and then stirred up for 2 hours on a magnetic stirrer, afterwards it will be centrifuged for 10 minutes (3500 rpm). Here supernatant is filtered and at -18 °C stored. 1 mL sample is added to 5 mL of 0.25 N Folin Ciocalteu reagent. Then 3 minutes incubation is done, 4 mL of 7.5% Na_2CO_3 is mixed to solution and incubation for 2 hours is done at room temperature. The absorbance will be observed at 765 nm. The results were expressed as mg of Gallic acid equivalents per 100 grams (mg GAE/100g)

b) Determination of Total Flavonoid Content (TFC)

The process involved mixing 50 µL of the methanolic extract with 180 µL of distilled water and 20 µL of a solution of 10 g/L 2-aminoethyldiphenylborate in a 96-well microtitration flat-bottom plate. The absorbance of the mixture will be then measured at 404 nm using a spectrophotometer. The flavonoid content is determined by comparing the absorbance of the extract to a standard solution of Rutin at various concentrations ranging from 0 to 200 µg/mL. The results were expressed as mg of Rutin per gram of dry weight (mg eq. Rutin/g db.).

c) Determination of antioxidant

The antioxidant activity of the sample will be determined using the DPPH assay. 0.1 ml of the prepared extract solution is taken and dissolved in 3.9 ml of a 0.06 mM methanolic solution of DPPH. The solution will then vortexed for 20 seconds and placed in a B.O.D. incubator for 30 minutes at room temperature. The absorbance of the solution was measured at 517 nm by a UV-VIS spectrophotometer. The absorbance measured of the DPPH radical in the absence of the sample is taken as the standard, and methanol is taken as the blank solution. The lower the absorbance of the reaction mixture, the greater the free radical scavenging activity.

Functional properties

a) Determination of Total Soluble Solids (TSS): The experiment protocol will be followed according to (Makanjuola, O. M., & Alokun, O. A. (2019), with slight variation/ modifications.

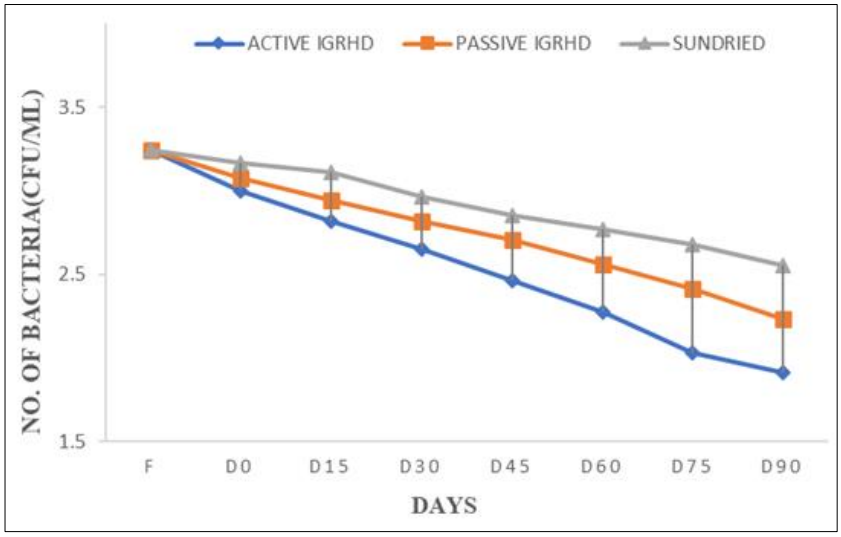
The TSS will be determined using an Abbe 60 refractometer corrected to 600C. The refractometer is set to zero using distilled water before use. A sample aliquot is placed on the

prism surface of the refractometer, and the total soluble solid is directly calculated as the sugar content.

b) Determination of pH

The pH of the samples will be determined using the AOAC, (2000) method with slight variation/modifications.

Result and Discussion



Keys: F= fresh sample, D0 = immediate drying sample, D15= drying the sample for 15 days, D30 = drying the sample for 30 days, D45 = drying the sample for 45 days, D60= drying the sample for 60 days, D75= drying the sample for 75 days, D90 = drying the sample for 90 days

Fig 1: Microbiology of bacteria

Microbiological Analysis

The results of the study (fig 1) showed that the active Infra-Red assisted Greenhouse drying method was the most effective in reducing bacteria growth. The decrease in bacteria was significant, compared to the other drying methods, such as the passive Infra-red assisted Greenhouse drying method and sun drying method. The passive IR drying method also demonstrated a decrease in bacteria, although it was not as pronounced as the active IR drying method. Meanwhile, the sun drying method had the lowest reduction rate of bacteria, making it the least effective method in reducing bacterial growth.

Also, the microbiological analysis revealed that strawberries dried under sun drying, active IR drying, and passive IR drying were free of coliform bacteria growth. This implies that the drying processes effectively prevented the spread of these harmful bacteria, ensuring the safety and quality of the dried strawberries.

Antioxidant Analysis

The antioxidant properties (fig 2) of dried strawberries were assessed using total phenolic content (TPC), total flavonoid content (TFC), and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) values. Strawberry sample dried under Infra-red assisted Greenhouse drying have superior quality as compared to other drying methods having highest total phenolic content (17.43±0.0)mg GAE/100g and lowest was observed in sun drying sample (16.53333±0.057735) mg GAE/100g. The highest total flavonoid content was obtained in sample dried under Infra-red assisted Greenhouse drying (3.346667±0.020817mg/100g) and the lowest in sample dried under sun drying (1.43±0.01mg/g). There was a corresponding drop in DPPH. The sun-dried samples had the greatest decrease, followed by the passively dried samples. The samples dried using the Infrared (IR) assisted active drying method had the least reduction. These findings demonstrate that the IRGHD approach efficiently retained the antioxidant capabilities of dried strawberries.

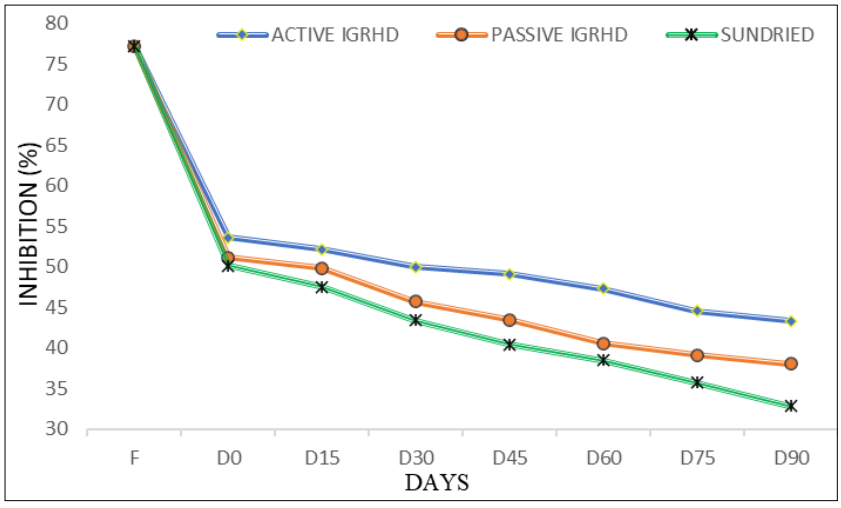


Fig 2: Total antioxidant capacity

Functional analysis

The highest Total Soluble Solid content was obtained in sample dried under Infra-red assisted active Greenhouse drying (10.31333 ±0.015275mg/100g) and the lowest in sample dried under sun drying (9.03±0.03mg/g). In the case of sun-dried, active IR-dried, and passive IR-dried strawberries, the results revealed a noticeable decrease in pH levels as the drying process progressed. This increase in acidity was reflected in a decrease in pH, with the most significant drop observed in the active IR-dried samples, followed by the passively IR-dried samples, and finally the sun-dried samples. The results show a significant change in the acidic balance of the dried strawberries.

Table 1: Total Soluble Solid (TSS)

Days	Active ighrd(◦brix)	Passive ighrd(◦brix)	Sundried (◦brix)
F^{0}	$11.53667 \pm 0.020817^{Aa}$	$11.53667 \pm 0.020817^{Aa}$	$11.53667 \pm 0.020817^{Aa}$
F	11.23 ± 0.02^{aB}	$11.02333 \pm 0.025166^{bB}$	10.77 ± 0.01^{cB}
D^{15}	10.97 ± 0.026458^{aC}	10.77 ± 0.026458^{bC}	$10.53333 \pm 0.020817^{cC}$
D^{30}	$10.82067 \pm 0.010066^{aD}$	$10.54667 \pm 0.025166^{bD}$	$10.23333 \pm 0.030551^{cD}$
D^{45}	$10.62333 \pm 0.015275^{aE}$	10.37 ± 0.112694^{bE}	9.85 ± 0.02^{cE}
D^{60}	10.528 ± 0.023065^{aF}	$10.14333 \pm 0.030551^{bF}$	$9.543333 \pm 0.030551^{cF}$
D^{75}	$10.43333 \pm 0.020817^{aG}$	$9.873333 \pm 0.015275^{bG}$	$9.343333 \pm 0.030551^{cG}$
D^{90}	$10.31333 \pm 0.015275^{aH}$	9.54 ± 0.200749^{bH}	9.03 ± 0.03^{cH}

Values expressed are Mean ± SD (n=3)

Conclusion

Finally, the research revealed that the active Infrared (IR) drying method is a highly effective method for drying strawberries. When compared to sun drying and passive mode greenhouse solar drying, this method demonstrated faster drying times. Furthermore, the active IR drying method was found to be superior in terms of preserving the antioxidant properties of strawberries, including Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Soluble Solids (TSS). The active IR drying method had the greatest impact on bacterial growth reduction, resulting in dried strawberries free of faecal contamination and safe for consumption. These findings highlight the potential benefits of using the active IR drying method to preserve strawberry quality and extend shelf life.

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