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Conventional and Enzyme-Assisted Extraction of Total Flavonoids from Rotten Onion (*Allium cepa* L.)

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ABSTRACT

One of the richest sources of dietary flavonoids is the bulb of the onion (*Allium cepa L.*), which greatly contributes to the daily intake of flavonoids. This research aimed to optimize the yield of Total flavonoids from Onion Solid Waste (OSW). A comparison was drawn between Conventional and Enzyme Assisted extraction methods in terms of the yield of total flavonoids present in OSW extract. The results showed that the highest TFC were obtained at Time/Temp 60 °C for 180 minutes and Pectinase Conc./Time 2g for 120 minutes for Conventional and Enzyme assisted extraction respectively. The yield of TFC by both methods showed that the yield for both was higher in case of enzyme assisted extraction for T13, i.e., 0.472 mg/g respectively than that for conventional extraction for T4 i.e., 0.352 mg/g.

Key words: *Onion Solid Waste, Conventional Extraction, Enzyme-assisted extraction, Total Flavonoids, Pectinase.*

Introduction

One of the key elements in Indian cuisine is the onion (*Allium cepa* L). Celano *et al.* (2021) rank it as the second-most significant horticultural crop, right behind tomatoes. In India, onions are a crucial export product that generates foreign cash. Onion production is rising globally, with China and India following closely behind. According to FAOSTAT, 65,430,064 tonnes of onions were produced worldwide in 2019. Increasing demands and production of onions have begun generating increased amounts of onion by-products during processing, such as peel/skin, and their disposal poses a burden on the environment. Onion skin and peels, however, are rich in bioactive substances including polyphenols and flavonoids, which encourages the use of these byproducts as functional additives. Onion is a vegetable that belongs to family Alliaceae, is cultivated in all parts of the world, is widely distributed, and grown in temperate, tropical, and sub-tropical climatic areas (Fritsch and Friesen, 2002). Onion has a characteristic aroma and flavor and has many medicinal and nutritional properties. It contains a variety of biologically active compounds viz. flavonoids, phenols, flavonols, anthocyanins, phenolic acids etc. Fossen and Andersen (2003).

Isoflavonoids, flavanols, flavones, flavonols, and anthocyanidins are the six subcategories of flavonoids. Flavonoid quercetin is a promising candidate for anticancer therapy since it can slow the growth of a variety of cancer cells. According to Bakht *et al.,* (2014), it exhibits antioxidant and/or prooxidant behaviour and affects many intracellular signalling cascades that are important to the organism.

Allium extracts have insecticidal Meriga *et al.,* (2012), antimicrobial Lawal *et al.,* (2016), anticancer Abotaleb *et al.,* (2019); Sak (2014) activities that are reported in several studies. An abundant source of

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phytochemicals and antioxidants, onions have several health advantages. Onions' various developmental stages' phytochemical contents and the enzymes involved in their manufacture have not been thoroughly researched. Flavanol synthase expression levels at various developmental stages and in diverse plant sections have not been investigated. There hasn't been any research done yet on the cytotoxic effects of various solvent extracts of native onion cultivars on various cell lines. These bioactive substances are said to offer a wide range of therapeutic and health-improving properties. Fresh green onions can affect the development and progression of cancer because they affect the pathways involved in cell proliferation, apoptosis, and metastasis. The primary green food item with active ingredients that have antioxidant, cytotoxic, and pro-apoptotic effects is the onion.

Enzyme assisted extraction (EAE), which often produces fewer undesirable compounds and has a reduced environmental impact, can be a viable alternative method. Cell walls are disintegrated by this high bioactive yielding method, allowing desired bioactive compounds to be released. To increase system transparency, it also eliminates extraneous elements from cell walls and barriers to water solubility and insolubility. It benefits from great catalytic efficiency and highly maintains the natural goods' original potency Jeon *et al.* (2011).

This study aims to compare and maximizing the efficiency of extracting the flavonoids from rotten onion through conventional and enzyme-assisted extraction.

Materials and Methods

Sample preparation

The onion waste was collected from market in Prayagraj. The onions were washed and then cut into uniform sizes. The cut onions were dried in Tray drier at 60 °C for 8 hours. The dried sample was taken out and ground into fine powder and then sieved to remove impurities. The fine powder was packaged in LDPE bags and stored at refrigeration temperature until used for treatment. 5g of samples was taken for extraction by both methods.

Formulation of various samples for conventional and enzyme assisted extraction methods

In case of Conventional extraction, nine samples (T0, T1, T2, ——, T9) were taken at SSR ratio of 1:20 at 50, 60 and 70°C for 120, 180 and 240 minutes respectively. The solvent for the process was chosen as Ethanol and Water (50% by volume) and was carried out in a Soxhlet extractor.

In case of Enzyme Assisted Extraction, nine samples (T9, T10, T11, ——, T17) were taken at different concentrations of pectinase enzyme viz., 1.5, 2 and 2.5 g at 40 °C for 90, 120 and 150 minutes respectively.

Yields of the Total Flavonoids

The yield of the total extracted flavonoids was calculated by the following equation given by Nan *et al.,* 2019.

$$\text{Flavonoid Yield (mg/g)} = \frac{c \times v \times n}{m} \times 4$$

Results and Discussion

The various findings of the present study are presented in tables below with corresponding bar graphs showing the trend of results obtained. The result was subjected to Two-way ANOVA with replications, and it was found significant.

The Two-Way ANOVA with replication showed that the treatments are found Significant at 1% and 5% level of significance CD (0.01) = 0.043 CD (0.05) = 0.031. Coefficient of Variation = 2.108

It is observed that the highest TFC was obtained at 60 °C for 180 minutes i.e., 0.098 mg QE/g in T4 while lowest value was observed at 70 °C for 240 minutes, i.e., 0.46 mg QE/g in T8. The results corroborated the work of Roldán-Marín *et al.,* 2009a, 2009b, 2010; Benítez *et al.* (2011) and indicated that the Total flavonoids compounds decreased as temperature increased.

The Two-Way ANOVA with replication showed that Treatments found Significant at 1% and 5% level of significance CD (0.01) = 0.004 CD (0.05) = 0.003 Coefficient of Variation = 2.356.

It is observed that the highest TFC was obtained at 2g for 120 minutes, i.e., 0.132 mg QE/g in T13 while lowest value was observed at 1.5g for 150 minutes, i.e., 0.035 mg QE/g in T15. Our findings were consistent with those published for celery seeds Sowbhagya *et al.* (2010), cumin seeds Sowbhagya *et al.* (2011), and garlic cloves Sowbhagya *et al.* (2009), when compared to earlier investigations, Fructus forsythiae, thyme, and rose-

mary Hosni *et al.,* (2013). It was also examined the effectiveness of each enzyme, concluding that pectinase pre-treatment produced the highest yields, which is consistent with our findings.

The result was found to be high Total Flavonoids in case of Enzyme-Assisted Extraction than Conventional extraction method. For both methods is presented in Tables 1 and 2.

The results demonstrated that the Total Flavonoid yield for T4 at 60 °C for 180 minutes in conventional extraction method was the highest, i.e., 0.352 mg/g, whereas the Total phenolic content yield for T6 at 50 °C for 240 minutes was the lowest in conventional method i.e., 0.192 mg/g. The research done by Nan *et al.* (2019) is in support of these findings. They argued that the average movement speed of a material towards a molecule would increase with increasing temperature, increasing the diffusion rate. The infiltration of plant tissue and the dissolving of useful substances, which might agglomerate proteins in plants and kill enzymes to assist the leaching of chemicals, were also encouraged by the rise in temperature.

The results demonstrated that the Total Flavonoid yield for T13 at 2g of pectinase for 120 minutes in enzyme assisted extraction method was the

Table 1. Total Flavonoids by Conventional Extraction Treatment

T0	T1	T2	T3	T4	T5	T6	T7	T8	
R1	0.065	0.077	0.067	0.082	0.097	0.093	0.054	0.060	0.044
R2	0.063	0.074	0.068	0.083	0.096	0.092	0.053	0.062	0.044
R3	0.058	0.078	0.069	0.081	0.098	0.091	0.054	0.061	0.046
Mean	0.063	0.078	0.069	0.082	0.098	0.093	0.054	0.062	0.046
F-test	S								
F				1.022					
FCritical				0.382					
CD(P=0.05)				0.031					

Table 2. Total Flavonoids by Enzyme-Assisted Extraction Treatment

T9	T10	T11	T12	T13	T14	T15	T16	T17	
R1	0.072	0.123	0.057	0.106	0.130	0.092	0.035	0.056	0.045
R2	0.072	0.117	0.055	0.105	0.132	0.093	0.035	0.052	0.044
R3	0.070	0.120	0.053	0.103	0.133	0.094	0.037	0.050	0.046
Mean	0.072	0.120	0.055	0.105	0.132	0.093	0.035	0.052	0.045
F-Test	S								
F	1.28								
FCritical	0.305								
CD(P=0.05)	0.040								

Table 3. Flavonoid Yield by Conventional Extraction

Sample	c (mg/ml)	v (ml)	n	m(g)	Yield (mg/g)
T0	0.063	0.5	9	5	0.226
T1	0.078	0.5	9	5	0.280
T2	0.069	0.5	9	5	0.248
T3	0.082	0.5	9	5	0.292
T4	0.098	0.5	9	5	0.352
T5	0.093	0.5	9	5	0.332
T6	0.054	0.5	9	5	0.192
T7	0.062	0.5	9	5	0.22
T8	0.042	0.5	9	5	0.148
F-Test	S				
F	0.567				
Fcritical	0.578				
CD(p=0.05)	0.022				

Table 4. Flavonoid Yield by Enzyme-Assisted Extraction

Sample	c (mg/ml)	v (ml)	n	m(g)	Yield(mg/g)
T9	0.072	0.5	9	5	0.256
T10	0.120	0.5	9	5	0.432
T11	0.55	0.5	9	5	0.136
T12	0.105	0.5	9	5	0.376
T13	0.132	0.5	9	5	0.472
T14	0.093	0.5	9	5	0.332
T15	0.035	0.5	9	5	0.124
T16	0.052	0.5	9	5	0.184
T17	0.045	0.5	9	5	0.18
F-test	S				
F	1.248				
Fcritical	0.313				
CD(p=0.05)	0.011				

highest, i.e., 0.472 mg/g, whereas the Total phenolic content yield for T15 at 1.5g of pectinase for 150 minutes was the lowest in enzyme assisted extraction i.e., 0.124 mg/g. These results were supported by the work done by Chen *et al.,* 2011 who explained the extraction yield increased to 1.3 times by treatment with pectinase resulting from its facilitating effect on cell wall degradation.

As it is clear from Tables 3 and 4 that the flavonoid yield was greater in EAE than CE, which is supported by the work done by Nan *et al.,* 2019. This comparative difference in both yields could be due to the loss of flavonoids during the temperature rise in CE method.

Conclusion

Long-term animal experiments had proved that excessive use of oxidants could cause irreversible damage to the body. Therefore, people began to pursue green and healthy natural antioxidants, such as flavonoids, alkaloids, and unsaturated hydrocarbons. This research aimed to optimize the yield of Total flavonoids from Onion Solid Waste (OSW). Enzyme Assisted Extraction was proven to be best amongst the two methods chosen for the extraction of bioactive compounds and gave a higher total flavonoid yield. It is intriguing to consider using enzymes to completely extract bioactive substances without the need of solvents. Enzyme pre-treatment of raw materials typically reduces extraction time, minimises the need for solvents, and increases yield and product quality. Prior knowledge of the cell wall composition of the raw materials helps in the selection of an enzyme or enzymes useful for pre-treatment. Reduced solvent consumption during extraction is crucial for environmental and regulatory reasons, offering a "greener" alternative to conventional non-enzymatic extraction.

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