International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 15, Issue 2, 2023, pp.-2016-2018.
Available online at https://bioinfopublication.org/pages/jouarchive.php?id=BPJ0000234

Research Article

MALDI-TOF MS IDENTIFICATION OF SPOILAGE YEASTS IN CASHEW APPLE (*Anacardium occidentale* L.)

GAYATHRY G.[1]*, MAHESHWARI P.[2], ARTHEE R.[3], SANGEETHA A.[4] AND JOTHILAKSHMI K.[5]

[1]ICAR-Krishi Vigyan Kendra, Vridhachalam, 606001, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India
[2]School of Agriculture and Animal Sciences, Gandhigram Rural Institute (DTBU), Gandhigram, Dindigul, 624302, Tamil Nadu, India
[3]ICAR-National Research Centre for Banana, Trichy, 620102, Tamil Nadu, India
[4]Department of Plant Pathology, Anbil Dharmalingam Agricultural College and Research Institute, Trichy, 620009, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India
[5]Department of Human Development and Family Studies, Community Science College and Research Institute, Trichy, 625104, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India
*Corresponding Author: Email - gayathryg@tnau.ac.in

Received: May 28, 2023; Revised: June 25, 2023; Accepted: June 27, 2023; Published: June 30, 2023

Abstract- Cashew apple is a rich source of sugars and vitamins that is discarded as a waste during cashew nut processing. Cashew apples starts fermenting soon after the harvest and due to the presence of wide spectrum of polyphenols and tannins, change of colour of cashew apples lead to an unappealing marketability and storage stability. The present study is aimed to rapid identification of different spoilage yeasts and to identify them using MALDI-TOF Mass Spectroscopy. Spoilage yeasts like *Candida krusei, C. tropicalis, Pichia norvegensis, Brettanomyces bruxellensis* were the spoilage yeasts found to high confidence score values of 2.00 - 3.00 with high consistency.

Keywords- *Cashew apple, MALDI-TOF MS, Spoilage yeasts*

Citation: Gayathry G., *et al*., (2023) MALDI-TOF MS Identification of Spoilage Yeasts in Cashew Apple (*Anacardium occidentale* L.). International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 15, Issue 2, pp.-2016-2018.

Academic Editor / Reviewer: Dr Ranjana Hawaldar, Rejeesh R

Introduction

Cashew apple is an edible sugary rich pseudo-fruit that can be eaten as raw or mixed with fruit salads and is exploited commercially for ready to serve beverages, drinks, sweets, jams, pickles, candies, vinegar, wine, cajuina in Nigerian states such as Bissau, Canchungo, Ingore and Quinhamel [1] Fenny in Goa, India and in several other cashew growing countries across the world. But in the international market, cashew apple-based products are less widespread. Cashew apples starts to ferment within few hours after harvest. The fleshy fruit is thin skinned which may be red or yellow coloured depending upon variety, spongy, fleshy, juicy with sweet smell, slightly astringent and acidic in taste [2]. Even the juice from cashew apples darkens quickly after extraction due to the rapid oxidation of some of the flavonoids, polyphenols and organic acids making it unfavourable for commercialization by making it into an unappealing dark brown colour. It has been a challenging venture to preserve cashew apple juice in bottles and serve as fresh without adding any preservatives.

Saccharomyces cerevisiae, the beneficial brewer's yeast is used in the mass production of cashew wine and renders good prospects for the alcoholic beverage sector. Fermented alcoholic beverage production with certain strains of yeast such as *S. cerevisiae* and *Hansenula guilliermondii* with cashew apple juice as the fermentative substrate, possess the capability to metabolize polyphenols and certain aroma compounds, such as acetate ester and β-phenylethanol during the process of fermentation [3]

Rapid analysis of bio-molecules/microorganisms based on the protein's patterns present in any live sample can be carried out through Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS). It a high throughput and rapid tool for species classification because of its, minimal pre-sample preparation, and displays high dynamic ranges for the organism identified.

Most importantly, MS allows for the detection of a wide microbial protein spectrum without the use of standard specific DNA primers or antibodies. These protein patterns serve as fingerprint region that could be constructed as yeast/bacteria/fungi or mold for genus and species classification of microorganisms. Currently, most published studies of the direct mass spectrometric analysis of microorganisms are based on MALDI techniques because of its speed and simplicity. There is only handful of studies for identifying yeasts in cashew apple fruit samples by MALDI-TOF MS.

Materials and Methods

Cashew apple juice extraction and isolation of spoilage yeasts

Cashew apple juice was obtained through a mechanical press in which the peeled cashew apples are compressed and squeezed of the juice and filtered using a cheese cloth. From this juice sample, one ml was inoculated into a sterilized Yeast Extract Malt Extract (YM) broth. Surface of the broth in the tubes were sealed with one cm layer of sterile paraffin and incubated at 28ºC for 48 h 9 [4]. One ml of incubated YM broth was taken and serially diluted up to 10-6 dilution in sterile water and one ml of 10-6 diluted suspension was spread over Yeast Extract Peptone Dextrose (YPD at pH 3.5) agar plates and incubated at 28ºC until yeast colonies appeared [5].

Purification and characterization of spoilage yeasts in cashew apples

After incubation, single yeast colonies, based on the well-separated, distinctness of colony morphology, isolates were selected, labelled, and purified by cross streak method on the YPD agar plates and incubated at 28ºC for 48 h.

Purification was repeated until to get morphologically identical distinct colonies. After the incubation period, the cultures were stored inside the refrigerator at 4ºC.

Table-1 Identification of spoilage yeasts in cashew apples by MALDI-TOF MS

SN	Identified spoilage organisms	Confidence of Identification	Consistency	Average Score value
1	*Candida krusei*	+++	A	2.59
2	*Candida tropicalis*	+++	A	2.12
3	*Pichia norvegensis*	+++	A	1.99
4	*Brettanomyces bruxellensis*	+++	A	1.93

The isolated yeast cultures were identified based on their cell morphology, colony characters and biochemical characters [6,7]. The yeast isolates were activated by inoculating each strain in a tube containing 25 ml of YPD broth containing yeast extract 1% (w/v), bacto peptone 2% (w/v), glucose 1% (w/v) and agar (20 g/L) and incubated at 28ºC for 24 h. After 24 h, physiologically active cells were used as inoculum for biochemical/physiological and for MALDI TOF MS identification tests.

Preparation of culture samples for MALDI-TOF MS identification

The yeast cultures isolated from the cashew apple extract was prepared as per the method developed by Schulthess *et al.* (2013) [8]. Direct colony transfer - formic acid method was adopted for preparing the isolated yeast cultures in feeding the MALDI-TOF MS. Young 24 hours grown yeast isolates (CAP 1 to CAP 9) were smeared separately on a polished steel MSP 96 target plate using a toothpick on which 1 µL of 70% formic acid was added and thin smear was prepared allowed for air drying. Followed by it, saturated-cyano-4-hydroxycinnamic acid (HCCA) matrix (1 µL) prepared using 50% acetonitrile and 2.5% trifluoroacetic acids solution was overlaid onto the air-dried yeast isolates on the target plate at room temperature. The target plate was placed into the plating chamber of the MALDI-TOF MS and shuttered for performing the identification of the yeasts.

Score values denotes

2.3 to 3.0 : highly probable species identification
2.0 to 2.3 : secure genus identification, probable species identification
1.7 to 2.0 : probable genus identification
0 to 1.7 : not reliable identification
'A : stands for high consistency

Brettanomyces bruxellensis is a ubiquitous yeast that possess the ability to form various volatile phenols and biofilm formation that confers to unpleasant organoleptic characteristics of wine and provoke economic loss for the wine industry. Due the production of volatile phenols, a stable or horse-sweat odour is liberated by *B. bruxellensis* that deters the quality of wine [9,10]. In earlier findings of sugarcane juice spoilage yeasts identification using MALDI-TOF by *Gayathry et al.,* 2022 [11], *Candida* was found to be predominating contaminant. In the present study also similar type of yeasts were identified, witnessing the off odour of the cashew apple heaps just in a day after harvest. Tournas *et al.* (2006) [12] reported that *Candida krusei, Pichia membranifaciens, Saccharomyces bisporus, Schizosaccharomyces pombe* and *Zygosaccharomyces bailii* were the spoilage yeasts in fruit juices. About 16 species of yeasts were identified by Vegas et al. (2020) [13] in which *Candida tropicalis, Debaryomyces hansenii, Hanseniaspora opuntiae, H. opuntiae* and *H. thailandica* were found to be abundant yeasts in native fruits of Amazon. Desai *et al.,* (2012) [14] identified five different species of *Candida* in cashew apple that tolerates high ethanol and sugar content. The results of the present identification of yeasts in cashew apples exhibited two types of *Candida* species namely C. krusei and C. tropicalis. Barros *et al.,* (2012) [15] isolated second-generation ethanol producing elite yeast genus namely *Hanseniaspora* in cashew apple fermentation medium. In the cashew apples used in the present study, *Brettanomyces bruxellensis* that were earlier reported by Mukadam *et al.,* (2016) [16] to produce kombucha in tea fermentation had been identified.

Conclusion

The results proved that there is inherent yeast colonization inside the fruits which proliferates upon time when sufficient nutrients are provided in the laboratory. Furthermore, spoilage yeasts such as *Candida*, *Pichia* and *Brettanomyces* are the contaminant candidate in preparation of value-added products from cashew apple rendering to early spoilage of fruits. Cashew apples should be processed immediately after harvest and can be valorised as a renewable and sustainable sugary rich source for the development various fermentative derived cashew apple byproducts. The spoilage yeasts identified from cashew apples may be exploited for the developed of high-quality wine or other yeast based fermented foods.

Application of research: Key spoilage organism in cashew apple processing

Research Category: Agriculture microbiology

Abbreviations: MALDI TOF-MS- Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry,

Acknowledgement / Funding: Authors are thankful to ICAR-Krishi Vigyan Kendra, Vridhachalam, 606001, Tamil Nadu Agricultural University, Coimbatore, 641003, Tamil Nadu, India; School of Agriculture and Animal Sciences, Gandhigram Rural Institute (DTBU), Gandhigram, Dindigul, 624302, Tamil Nadu, India; ICAR-National Research Centre for Banana, Trichy, 620102; Department of Plant Pathology, Anbil Dharmalingam Agricultural College and Research Institute, Trichy, 620009; Department of Human Development and Family Studies, Community Science College and Research Institute, Trichy, 625104, India

****Principal Investigator or Chairperson of research: Gayathry G.**
University: Tamil Nadu Agricultural University, Coimbatore, 641003, India
Research project name or number: Research station study

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: ICAR-Krishi Vigyan Kendra, Vridhachalam, 606001

Cultivar / Variety / Breed name: Cashew apple *Anacardium occidentale* L. VRI 3

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Mohanty S., Ray P., Swain M.R. and Ray R.C. (2006) *Journal of Food Processing and Preservation*, 30, 314-322.
[2] Catarino L., Menezes Y. and Sardinha R. (2015) *Scientia Agricola*, 72, 459-467.
[3] Gamero A., Ren X., Lamboni Y., de Jong C., Smid E.J. and Linnemann A.R. (2019) *Fermentation*, 5, 71.
[4] Gupta P.K., Garg S.K. and Mital B.K. (1994) *Beverage Food World*, 20-28.
[5] Pelczar M.J. and Reid R.D. (1958) *Microbiology. Krishna Prakashan Media.*
[6] Kreger-Van Rij N.J.W. (1984) *The Yeast a Taxonomic Study. Elsevier Science Publisher BV, Amsterdam,* 1082.

[7] Mesa J.J., Infante J.J., Rebordinos L. and Cantoral J.M. (1999) *Lebensmittel-Wissenschaft und-Technologie*, 32, 114-120.
[8] Schulthess B., Brodner K., Bloemberg G.V., Zbinden R., Bottger E.C. and Hombach M. (2013) *Journal of Clinical Microbiology*, 51, 1834-1840.
[9] Agnolucci M., Tirelli A., Cocolin L., and Toffanin A. (2017) *World Journal of Microbiology and Biotechnology*, 33(10), 180.
[10] Dimopoulou M., Renault M., Dols-Lafargue, Albertin W., Marie H.J., Marie-Noelle B.F. and Isabelle M.P. (2019) *Annals of Microbiology*, 69, 1217-1225.
[11] Gayathry G., Maheswari P., Jagadeesan A.R., Kalai Selvi R. (2022) *YMER*, 21 (8), 1343-1352
[12] Tournas V.H., Heere J. and Burgess L. (2006) *Food Microbiology* 23(7), 684-688
[13] Vegas C., Zavaleta A. I., Canales P. E., and Esteve-Zarzoso B. (2020) *Polish journal of microbiology*, 69(3), 1-11.
[14] Desai M.V., Dubey K.V., Vakil B.V. and Ranade V.V. (2012) *International Journal of Biotechnology for Wellness Industries*, 1, 259-265
[15] Barros E. M., Rodrigues T. H., Pinheiro A. D., Angelim A. L., Melo V. M., Rocha M. V. and Gonçalves L. R. (2014) *Applied biochemistry and biotechnology,* 174(8), 2762-2776.
[16] Mukadam T.A., Kapil P., Sunita D.D., Vaidya S.P and Chowdhary A.S. (2016) *International journal Current Microbiology and Applied Sciences*, 5(6), 32-41.