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Impact of Transcriptomics on Food Quality: A Comprehensive Approach

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Abstract

Transcriptomics holds significant importance in comprehending the genetic regulation of various processes in food. The analysis of the complete set of RNA transcripts produced by the genome under specific conditions is referred to as Transcriptomics. It involves the use of high throughput technologies i.e. Microarray, RNA sequencing, and RT-PCR. In the context of food, transcriptomics has a vivid role in food safety, nutritional enhancement, microbiome analysis, food processing, and preservation. It provides a molecular level of information on gene expression which enhances decision-making and innovations in food-related research and development. Transcriptomic data can be used to integrate into personalized nutrition. The gene expression can monitor the chances of contamination in food processing and storage. This review will highlight how significantly transcriptomics is contributing to the various aspects of the food industry.

Keywords: transcriptomics, RNA, RT-PCR, gene-expression

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Introduction

Foodomics-based approaches included several strategies to enable comprehensive characterization of the ingredients of the meat, fish, milk, and dairy products and improve our understanding of potential mechanisms and molecules related to the quality traits. These strategies helped to produce high-quality food [1]. Foodomics involves many disciplines that integrate food science with nutrition [2]. Advanced techniques like transcriptomics, proteomics, metabolomics, nutrigenetics, microbiomics, and others can help us understand the complexity of the foodome. The foodome refers to all the compounds in a food sample or a biological system that interacts with the food being studied at a particular time. These techniques are essential tools in the field of foodomics [3]. Transcriptomics is one of these omics techniques that has helped us to understand how many factors might alter the patterns of gene expression [4]. The transcriptome is the total set of RNA molecules that are expressed in a single organism at a certain moment. It is dynamic because it can change in response to different stimuli. The primary objective of transcriptome analysis, which involves three basic methods, is to find differentially expressed genes (DEGs) that differ among the situations under investigation [5]. The main objective of transcriptomics is to accurately and thoroughly characterize the transcriptome. This includes three main tasks: (1) measuring the amount of each transcript that is expressed differently under various conditions; (2) annotating all transcript types, such as messenger RNAs, non-coding RNAs like small and long non-coding RNAs, transfer RNAs, and ribosomal RNAs; and (3) figuring out the transcriptional structure of genes e.g. start sites, 5' and 3' ends, splice variants [6]. The transcriptome can be analyzed using three main methods: next-generation RNA sequencing (RNA-seq), microarrays, and real-time RT-PCR [7]. DNA microarrays consist of a condensed, well-organized configuration of single-gene nucleic acid fragments positioned at specific locations on a solid substrate. By using targeted hybridization to detect changes in hundreds of genes' expression in numerous samples concurrently, they are highly effective methods for determining the impact of various nutrients [8]. Since mRNAs serve as the mediator between genotype and phenotype in biological investigations, they are crucial for comprehending the molecular components of cells as well as the functional components of the genome. The stability of an organism's genome defines its transcriptome, which is dynamic and changes in response to a wider range of internal and external stimuli [9]. Real-time RT-PCR is the technology of choice for targeted investigations of certain genes because of its quantitative characteristics, which enable the identification of minute variations in gene expression across experimental settings [10]. When searching for a comprehensive perspective in untargeted investigations, RNA-seq is the preferred technology. Micro-arrays allow the investigation of almost the whole transcriptome if all the genes known to exist in a particular microorganism are included while being considered a focused approach. Thus, in an experimental setting, this strategy allows researchers to see changes in gene expression

[5]. The application of transcriptomics analysis in food is a relatively advanced development compared to their use in basic sciences.

Analytical Techniques

Microarrary

A microarray is a multiplex, high-throughput screening technique that places nucleotide probes in two dimensions on a tiny silicon chip or glass slide. mRNA helps in understanding any change in gene expressions. It is quite necessary to convert mRNA into cDNA which are labeled by fluorescent dyes Cy-3(green) or Cy-5 (red). To enable the identification of each fragment based on its location on the array, the many DNA fragments are arranged in a row and columns. There are two types of microarrays: tissue microarrays and gene expression microarrays. The target DNA fragments can be identified by their fluorescence emission by means of a laser beam (**Figure 1**) [11]. Using a computer, the fluorescence emission pattern and DNA identification are recorded. The DNA chip method is quite rapid [12]. In context of food microarrays can be employed for various purposes such as for food quality, for food safety for authentication of food, food allergen etc. While microarrays have been used extensively in the past, it's crucial to remember that, because of their increased sensitivity and capacity to identify novel transcripts, more recent technologies, such RNA sequencing (RNA-seq), are becoming more and more popular for gene expression analysis. Microarrays are still an economical and useful solution, nonetheless, in some food sector applications.

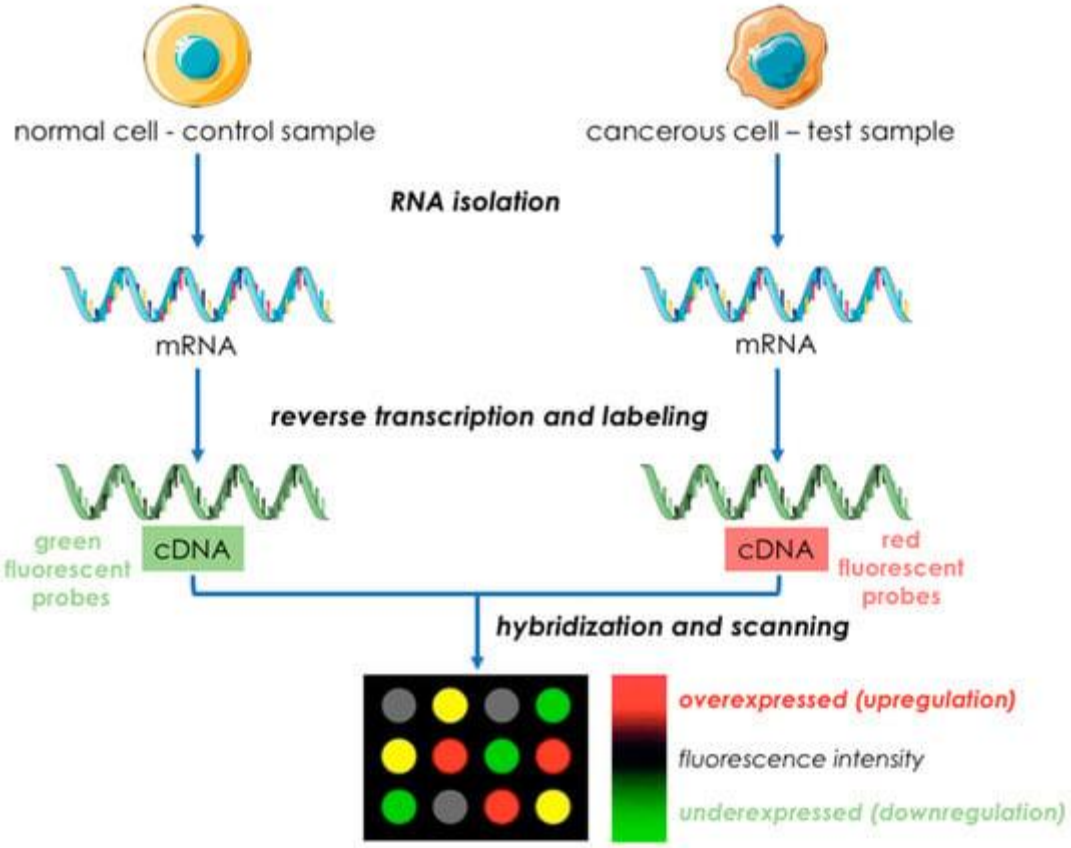

Figure 1 Identification by Microarray technique (Supplit *et al.*, 2021).

RNA-Sequencing

The other method known as “RNA sequencing” determines the amount and sequences of RNA in a sample. It analyses the transcriptome to which genes are turned on or off and to what extent. The term "RNA-Seq" describes the sequencing of cDNA transcripts, where the quantity of each transcript is determined by counting the number of transcripts [13]. The techniques used for RNA extraction are generally similar to each other, despite the extreme diversity of biological systems. This means that you can be confident in the results you obtain, knowing that the procedure has been tried and tested. So, whether you're conducting research or carrying out diagnostic testing, you can trust that RNA extraction techniques will provide you with accurate and consistent results. The process of

isolating RNA involves several steps, including the mechanical breakdown of cells or tissues, treatment with chaotropic salts to break down RNase, separation of RNA from unwanted biomolecules like DNA, and concentration of RNA through precipitation or elution. It is also possible to treat isolated RNA with DNase to break down any remaining DNA. To enrich messenger RNA, it is necessary to remove ribosomal RNA, which comprises 98% of total RNA extracts. This can be done through the use of sequence-specific probes or poly-A affinity techniques. It is important to note that damaged RNA can negatively affect downstream outcomes. For instance, 5´ mRNA ends will be depleted and there will be an inconsistent signal along a transcript's length when mRNA enrichment is performed on damaged samples. It is customary to snap-freeze tissue before RNA isolation, and once isolation is finished, precautions are taken to limit exposure to RNase enzymes [14] Over the last decade, there has been remarkable progress in next-generation sequencing (NGS) technologies. This advancement is due to the intense competition among manufacturers, leading to the development of various generations of platforms with improved capabilities for sequencing DNA at costs that were previously unachievable. NGS technologies are transforming the way we study biological systems. A typical RNA-Seq experiment involves the following steps: RNA isolation, cDNA conversion, library preparation for sequencing, and NGS platform sequencing (**Figure 2**) [15]. However, before conducting RNA-Seq, researchers need to consider several experimental factors based on their goals [16]. The steps involved in the analytical process are the same regardless of the NGS platform selected: (1) library preparation, which includes cDNA synthesis, ligation to specific adaptors at both ends and RNA molecule fragmentation; (2) clonal amplification of each template; (3) attachment of the amplified DNA templates to a solid support in a reaction chamber or flow cell; and (4) iterative and synchronized flowing and washing off the reagents for DNA strand extension while signals are acquired by the detection system [17]. Hence, RNA sequencing has great potential in food science, it is important for improving agricultural methods, comprehending the genetic basis of food-related features, and guaranteeing the quality, safety, and authenticity of food items. A significant amount of raw sequence reads from RNA-Seq experiments must be processed to produce meaningful data. Depending on the objectives and design of the experiment, a variety of bioinformatics software packages are typically needed for data analysis. Four stages comprise the process: differentiation expression, quantification, alignment, and quality control [18].

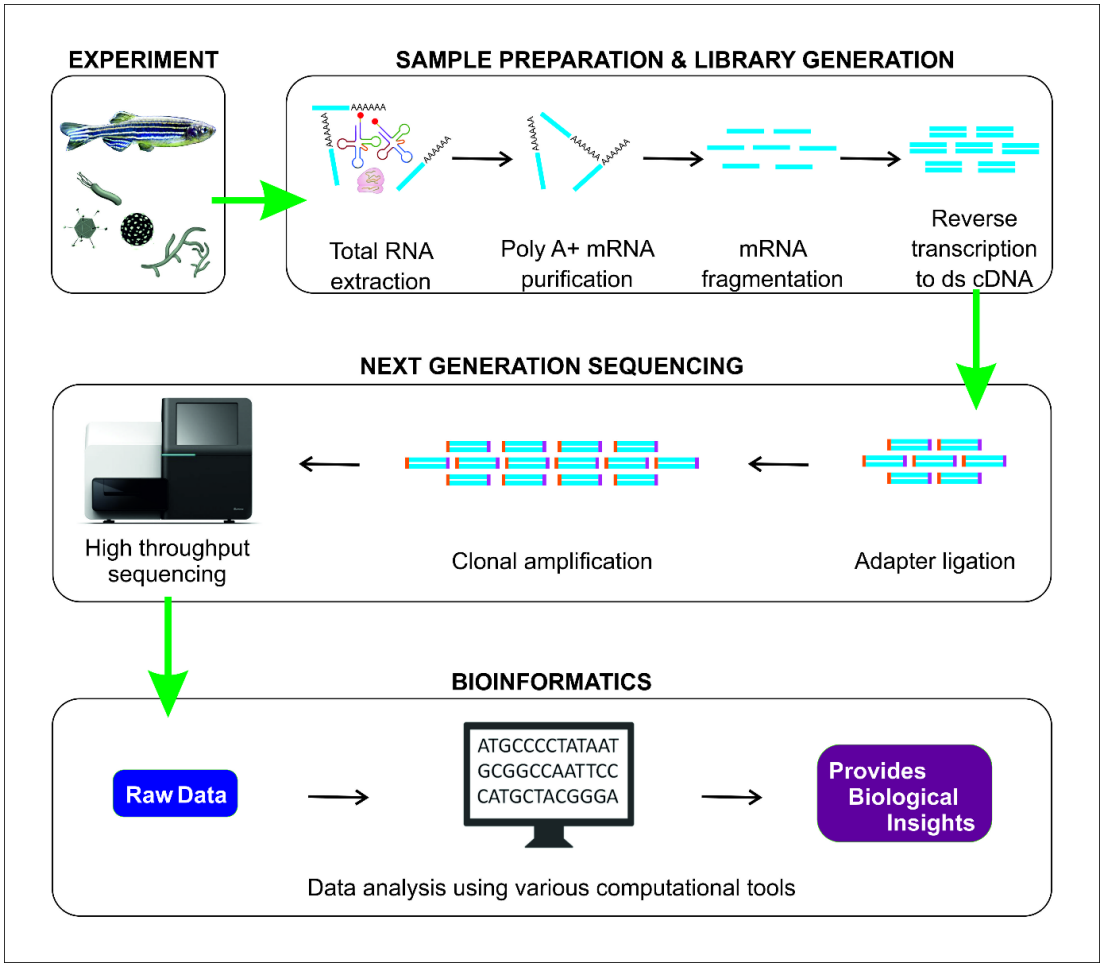

Figure 2 NGS -based RNA Sequencing (Sudhagar *et al*., 2018).

Transcriptomic Biomarkers

Specific RNA molecules or patterns of gene expression are known as transcriptomic biomarkers in food, and they can be utilized as markers of several factors about food safety, nutritional value, and quality. These biomarkers can be utilized for traceability, quality control, and even customized nutrition since they offer insightful information on the molecular processes that take place in food. It's crucial to remember that the population under investigation, the precise objectives of a study, and the type of dietary interventions can all influence the choice of these genes as biomarkers. High-throughput methods such as RNA sequencing or microarray analysis are frequently used by researchers to evaluate the expression of several genes at once and find putative transcriptomic biomarkers. The use of bioinformatics tools to analyze gene expression facilitates a more comprehensive comprehension of metabolic pathways and regulatory networks. This analysis helps in identifying biomarkers for diagnosis and prognosis, and also potential targets for medical and nutritional intervention. Transcriptomic studies have significantly enhanced our understanding of the intricate interplay between genetic and environmental factors, for instance, lifestyle and nutrition factors [19].

Table 1 Transcriptomics biomarkers which can be used as nutritional biomarkers for precision nutrition and optimal health (Pico *et al*., 2019) [20].

Transcriptomic Biomarkers	Biological Processes involved	Uses
PPAR-Alpha (PPARA)	Lipid metabolism	Used as biomarker in relation to the effects of dietary fats and fatty acids.
TNF-Alpha (TNF)	Inflammation	Changes in inflammatory expression can be studied
SIRT1	Longevity and metabolic responses	Studied in relation to caloric restriction and certain dietary compounds.
NRF2 (NFE2L2)	Antioxidant responses	Studied how diet affect cellular stress and antioxidant defense
FASN	Lipogenesis	alterations in lipid metabolism
SOD (Superoxide Dismutase) Family	Antioxidant defense.	Impact of diet on oxidative stress.
GLUT4	glucose metabolism and insulin sensitivity	carbohydrate metabolism and dietary carbohydrates.

Transcriptomics Contribution to the Food Sector

Transcriptomics is an effective tool in food science that offers a molecular-level understanding of several processes involved in quality control, flavor development, crop improvement, and food safety, among other applications.

Crop improvement

- **Yield improvement**: The identification of genes linked to desired characteristics in crops, such as nutritional value, disease resistance, and yield, depends heavily on transcriptomics. Breeding programs designed to create better crop types are guided by this information. RNA-seq has been used to study how postharvest conditions affect fruit quality. Two such instances are the sweetening of potatoes [21] and the loss of aroma in pears [22].
- **Stress Response**: Developing crop varieties that are resistant to stress is made possible by an understanding of the transcriptome under stressful environments, such as severe heat or drought. RNA-Seq analysis was performed on the exocarp of zucchini fruit at the time of harvest and after 14 days of storage at both 4 and 20 °C. The aim was to investigate the transcriptomic changes that occurred during this period. The results of this study identified several molecular mechanisms that may be associated with chilling tolerance. These mechanisms were either up-regulated in the cv. Natura (which is CI tolerant) or down-regulated in the cv. Sinatra which is CI-sensitive [23].

Food microbiology

- **Pathogen detection**: The application of transcriptomics can help in the development of quick and precise detection techniques by detecting and tracking the gene expression patterns of foodborne pathogens. These findings suggest that treating *B. siamensis* may impact several pathways, hence inducing disease resistance in

mango fruit. These discoveries establish a biological method to preserve the quality of post-harvest mango fruit in addition to illuminating the transcriptional regulatory mechanisms governing postharvest illness [24]. For many years, phenotypic investigations have served as the cornerstone in the microbiome of food, and they have made significant contributions to our knowledge of the behavior of foodborne pathogens in settings akin to those encountered in the food supply chain. Nevertheless, it clarifies which metabolic pathways are engaged in bacterial survival and which transcriptome technologies are required. It would be feasible to create more targeted biocidal medicines that target the primary pathways involved in bacterial survival and persistence by using the data from transcriptome investigations.

- **Microbial safety**: The application of transcriptomics can help in the development of quick and precise detection techniques by detecting and tracking the gene expression patterns of foodborne pathogens. Through a transcriptomic analysis, we have identified a set of genes responsible for *L. rhamnosus* growth in cheese during ripening by alternative metabolic pathways [25].

Food processing and Biotechnology

- **Fermentation optimization**: When it comes to producing particular foods like cheese, yogurt, and fermented drinks, transcriptomics is used to optimize the fermentation process. Fermentation processes can be made more regulated and efficient by studying the transcriptome of food-grade bacteria. Lactic acid bacteria (LAB) from 45 kimchi samples that had been fermented at temperatures between -1.5 to 0 degrees Celsius for 2 to 3 months. They found that *Weissella koreensis* strains were the dominant LAB in all the kimchi samples. However, no virulence traits were detected. The results showed that *W. Koreensis* strains are a promising culture for fermenting vegetables or fruits at low temperatures [26].
- **Enzymatic Browning**: Reports suggest that blocking the expression of the polyphenol peroxidase gene can reduce browning in potato tubers [27] and *Agaricus bisporus* [28]. Similarly, the activities of POD and PPO using synephrine hydrochloride (Syn-HCl) can reduce browning in litchi pericarp [29]. In a transcriptional study on recently cut eggplant fruit, including cultivars that were browning-resistant and browning-sensitive [30].
- **Food flavour and aroma:** Transcriptomics is a useful tool for finding genes related to aroma and flavour in food. These genes are produced by volatile molecules that are detected by the system. Producing food products with more flavor can be guided by an understanding of these pathways. The production of fruit volatile compounds, such as the "peach-like" aroma found in strawberries, has been studied using RNA [31]. In a separate study, it was found that Pear fruit aroma production is a complex process influenced by hormones, and that Methyl jasmonate (MeJA) therapy can enhance this process.They conducted complete-transcriptome RNA-sequencing (RNA-seq) of the peel and flesh of "Nanguo" pear treated with MeJA [32].
- **Optimizing Nutrient Levels**: Transcriptomics can identify the genetic variables affecting how important nutrients are synthesized in plants. To design crops with improved nutritional profiles, this information is useful. Children's consumption of high-fat (UCN2) or sugary food (TAS1R3) has been linked to variations in the expression of particular genes in plasma blood cells. Biomarkers are analytical indicators that may indicate the intake of a certain nutrient or food component and also reflect the effect of ingestion on the body. They can be used as short or long-term exposure indicators. These biomarkers can be quantitative [33]. Half of the selenium present in blood consists of proteins such as selenium transport and supply protein (SEPP) and glutathione peroxidases (GPX). These proteins are involved in antioxidant action. There is no regulated reserve pool of selenium in the body, so any excess selenium consumed above the body's requirements is expelled. Optimal selenium intake is linked to the optimal expression of selenoproteins [34]
- **Genetic Fingerprinting**: Food products can be authenticated and tracked back by transcriptomic profiles, which act as genetic fingerprints. This is particularly important when confirming the provenance and legitimacy of upscale or specialized foods. The identification of SSRs (simple sequence repeats) from the transcriptomes of two accessions: one *S. aethiopicum* and one *S. incanum* [35].The researchers selected SSRs based on their quality characteristics and further examined their coverage and length using the IGV (Integrative Genomics Viewer) software. As a result, they identified 11 highly reliable polymorphic SSRs [36].
- **Comprehending Individual Responses**: Transcriptomics plays a role in customized nutrition by providing insights into how people react genetically to different diets, enabling the customization of dietary recommendations Integrative nutritional biomarkers can provide valuable information about individual variations in diet response and help monitor responses to therapeutic interventions, making them more personalized. They can also be used to monitor effective intakes, validate or supplement intake

questionnaires, and shed light on physiological or pathological responses to specific food behaviours. These biomarkers could also play a significant role in creating individualized dietary guidelines, or "precision nutrition," aimed at achieving optimal health and wellness for specific phenotypes and genotypes [37].

- **Identification of Allergenic Proteins**: Genes and proteins linked to food allergies can be found using transcriptomics. This facilitates the development of techniques for identifying and managing allergens. The allergenicity of legume proteins and concluded that within a protein family, gene expression assay may be able to differentiate mildly from highly allergenic legume proteins; however, creating a universal technique for all protein families derived from plant and animal sources will be difficult [38].
- **Food Colour:** Transcriptomics has been used to study the pigment biosynthesis. More specifically transcriptomics has been to understand the biosynthesis of anthocyanins and carotenoids in fruit and vegetables. **Table 2** discuss the gene responsible for colour in some fruit and vegetable by specific genes is of importance [39].

Table 2 Analysis of Food colour from different fruit and Vegetables using Transcriptomics

Food Colour	Methodology	Results
Pepper Carotenoids	qRt-PCR, RNA Seq	In red and orange pepper fruits, there were notable differences in the CHY1 and CCS genes.
Jaboticaba anthocyanin	qRT- PCR, RNA Seq	Anthocyanin accumulation was favorably associated with genes related to ethylene and abscisic acid.
Cherry fruit Anthocyanin	qRT-PCR, transcriptomic Seq	The expression of the major structural genes related to anthocyanins, CHS, CHI, F3H, and F3′H, was stimulated by light.
Litchi anthocyanin	RNA- Seq	During storage, the anthocyanin-biosynthesis pathway is active in the litchi pericarp.

Conclusions

In conclusion, transcriptomics is a potent instrument in the food business that offers insightful information on the patterns of gene expression and the molecular mechanisms that underlie diverse processes. Its uses include boosting crop qualities, guaranteeing food safety, improving quality, and helping to create novel and sustainable methods of food production. Transcriptomics is a useful tool that can help detect and control foodborne pathogens. Personalized nutrition plans can also be created for individuals using this technology. By comparing gene expression profiles, unique transcriptomics signatures can be identified for specific food products, which can then be used for traceability and authentication. Additionally, transcriptomics can guide the development of microbial or plant-based food ingredients, such as flavors, colors, and nutraceuticals. These transcriptomics methods help to advance food science and technology research by providing a deeper comprehension of the molecular mechanisms underpinning many aspects of food safety, quality, and production.

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