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Biotechnological Utilization of Kluyveromyces (3 Strains) for Flavor Compound Production from Agricultural Wastes"

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Abstract: An emerging field of biotechnology that has strong implications for the environment and economy is the use of yeast strains to convert sustainable agricultural and dairy wastes into useful products. These yeasts have special metabolism which enables them convert low value substrates into high value flavors and fragrances through different fermentation processes. K. marxianus is capable of producing numerous kinds of volatile molecules due to its fast growth rate and thermotolerance while K. lactis as well as K.fragilis can produce specific compounds like volatile sulfur compounds or glycerol respectively when grown under certain conditions known from prior researches. In this review we describe what these microorganisms are able to do according on substrate specificity; process optimization methods employed during their utilization as flavor producers besides potential applications in biotechnology concerning flavors are also discussed here.

Keywords: Biotechnologica, Flavor Compound Production, Agricultural Wastes

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Introduction

Sustainable and eco-friendly ways for generating natural flavor compounds were sought due to customer desire for natural and healthier food items. Synthetic taste additions were cost-effective but may harm humans and the environment. Therefore, it was essential to find natural tastes that might replace synthetic ones (1).

This study investigated how three strains of Kluyveromyces yeast are utilized in biotechnology to extract natural flavor components from residues. The Kluyveromyces yeast group has shown carbohydrate metabolism in agricultural residues and byproducts. Leveraging the metabolic capabilities of these yeast strains we developed an eco economical method, for generating natural flavor compounds as substitutes, for additives (2)

Agricultural leftovers, like peels from fruits, scraps from vegetables and bran from cereals are known to hold reserves of carbohydrates and other important substances. Transforming these inexpensive raw materials into premium natural flavor components using Kluyveromyces yeasts offers a hopeful strategy to meet the increasing need, for eco friendly and sustainable flavor options. (3).

Materials and Methods

Kluyveromyces

1.1. Characteristics of the Species

Synthetic biology has expanded biotechnology into medical, farming, food, and chemistry. Increased sustainability and environmental awareness drive this expansion (4).

Careful design and construction may modify microbial chassis cells to utilize sustainable biomass as their substrate. Saccharomyces cerevisiae is famous for its involvement in green manufacturing, including natural goods, biofuels, and bulk chemicals. It also shows potential in lignocellulosic valorization (1).

Because of their adaptability to a wide variety of substrates, capacity for high-density aerobic fermentation, and tolerance to a broad pH and temperature range, Kluyveromyces (particularly K. marxianus and K. lactis) have shown significant promise as yeast chassis. Kluyveromyces bacteria, of which K. lactis is a species, are renowned for their safety and remarkable -galactosidase activity. Its suitability for the production of lactose-free dairy products is attributed to these attributes. (1) (Table 1).

Table 1. Industrially relevant products produced by Kluyveromyces sp (1).

Product	Description	Application
Lactase DENAZYME GY2	Enzyme preparation derived from Kluyveromyces, used to enhance aroma and facilitate fermentation in dairy products	Dairy processing
β-Galactosidase DS 80496	Lactose-reducing enzyme derived from K. lactis, used in specific dairy products	Dairy processing
Chymosin	Chymosin enzyme generated by K. lactis strains, used in cheese production	Dairy processing
Chymosin B	Chymosin B enzyme generated by K. lactis CIN strain, used in dairy product production	Dairy processing
FROOTZEN®	First commercially available product derived from Pichia kluyveri strains, used for incorporating natural flavorings into wine	Wine production
Complex microbial community	Feed additive derived from dried and incubated Kluyveromyces fragilis yeast, used to modify and regulate water quality, as well as for animal feed	Aquaculture, poultry, and cattle feed

A safe yeast, K. marxianus can grow at 52 °C and is safe for consumption. The biorefinery industry is interested in this yeast due to its quick growth, high tolerance, and flexibility in using affordable substrates (2) (Fig. 1).

Over the past few years, numerous companies have been exploring the application of different strains of Kluyveromyces yeast in various industries such as food, feed, and bioengineering. These efforts have yielded positive results, as evidenced by the successful development of several products, as detailed in Table 1. Exciting advancements are on the horizon for the utilization of Kluyveromyces in the coming decade (1).

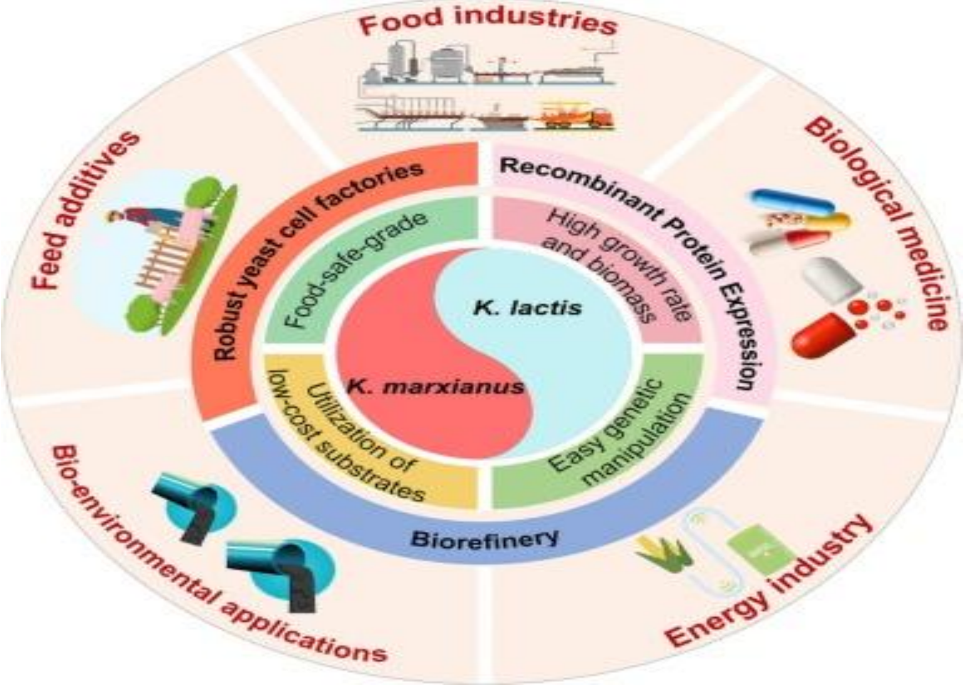


Fig. 1. Overview of the characteristics and uses of Kluyveromyces.

1.2. Characteristics that define the unconventional yeast species Kluyveromyces sp. in general.

The genus of Kluyveromyces sp. was initially named as a tribute to the renowned Dutch microbiologist Albert Jan Kluyver. With a rich research background (Fig. 2) (5), Kluyveromyces, a type of industrial yeast, has been the subject of substantial research. It is a naturally occurring substance that is detected in fruit trees, saltwater, and dairy products, among others. Historically, species classifications and diagnoses were mostly based on broad physical characteristics and biological activities. These included the formation of ascospores, nutritional requirements, ovoid and ellipsoid cell morphologies, and the emergence of pseudohyphae. The yeast cell is completely enveloped by a robust cell wall composed mostly of lipids, proteins, and polysaccharides. The main bioactive constituents are of α-Mannan and β-glucan. (5).

Kluyveromyces can be identified by 5.8 rRNA gene and two ITS sequences. Karyotype study categorizes Kluyveromyces species into two groups. One group has few chromosomes, while another, like Saccharomyces, has more than 12. Common species include K. marxianus and K. lactis (1).

1.2.1. Sexual reproduction and genomic features of Kluyveromyces sp.

Scientists have been able to get significant insights into the distinctive variances and common attributes of several strains of Kluyveromyces sp. because to the expanding compilation of sequenced genomes. As of yet, reports and functional annotations for 28 genomes of several Kluyveromyces species have been published. The GC contents of these 28 strains exhibited a typical range of 35.5 percent to 43.9 percent, although the genome length varied between 9.6 and 10.9 Mb. Complete sequencing of the genomes of Kluyveromyces marxianus and lactis has been completed, whilst the sequencing of the genomes of the remaining Kluyveromyces strains is ongoing (Table 2) (1).

The genome of K. lactis GG799 has six chromosomes and has a total length of 10.7 Mb. The GC content of the substance is 38.7%. Widespread use is made of this yeast strain to express foreign proteins **(Chuzel et al., 2017).**

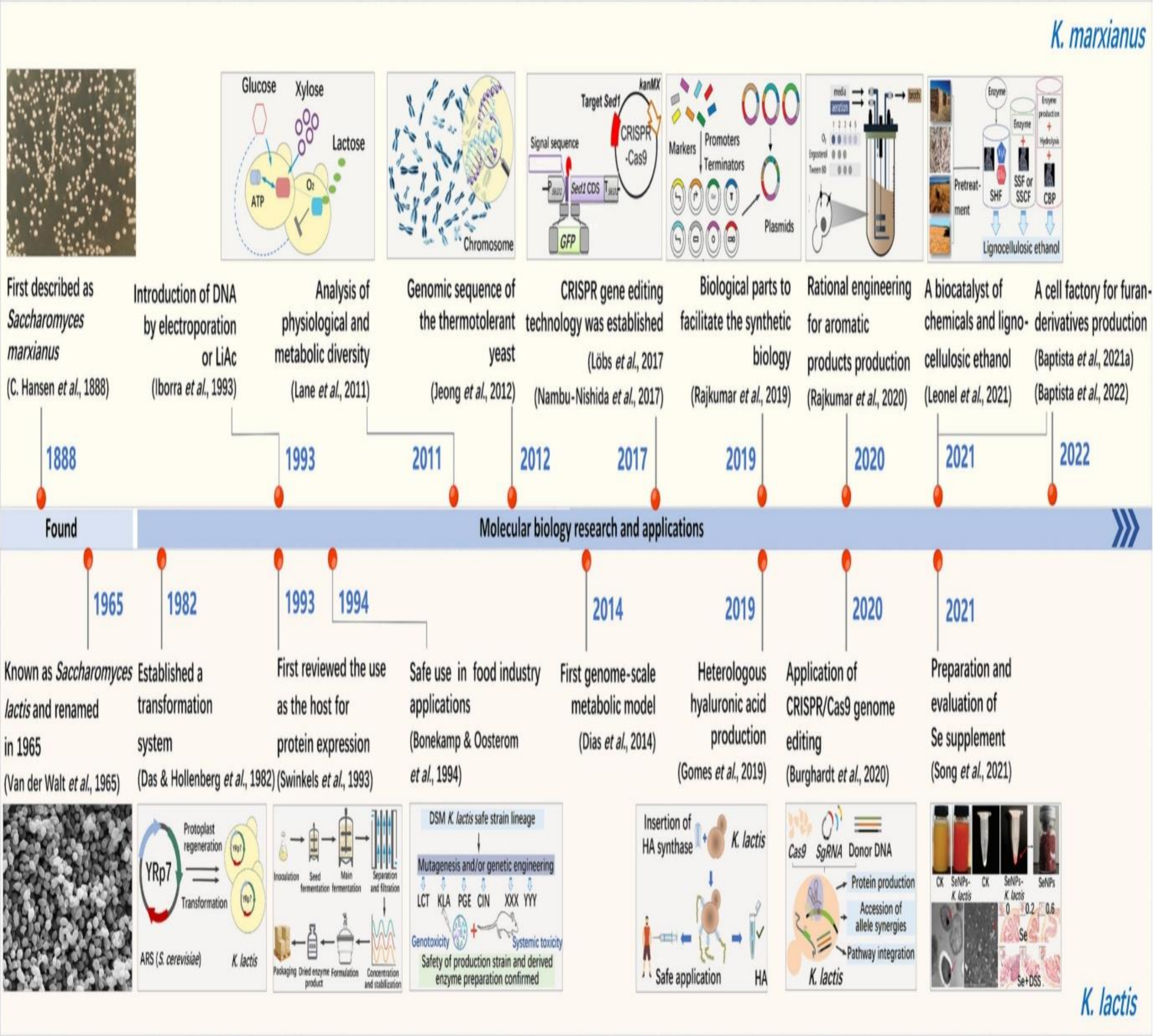


Fig. 2. Brief timeline of key milestones in the research history of *K. marxianus* and *K. lactis* (6-18)

Table 2. Representative host strains of Kluyveromyces sp. and well-annotated genomic information (1).

Strain	Culture Conditions	Key Characteristics

K. marxianus NBRC 1777	YM medium at 45°C, pH 6.0	Thermotolerant haploid with robust ethanol and cellulase production
K. marxianus FIM1	YG medium for 72 hours at 30°C, 220 rpm, pH 5.5	Originates from yogurt; notable for its high protein production capabilities
K. marxianus CBS 6556	YPD medium for 72 hours at 30°C, 300 rpm	Haploid; efficiently synthesizes inulinase or β-galactosidase
K. marxianus DMKU3–1042	Sugar cane syrup medium for 72 hours at 35°C, 300 rpm	Thermotolerant, suitable for high-temperature ethanol production
K. marxianus NRRL Y-6860	SSF from acid-treated rice straw at 45°C	Thermotolerant, potential for producing cellulosic ethanol
K. marxianus SLP1	Cultured under varying inhibitor conditions at 30°C, 250 rpm, pH 4.5	Diploid and thermotolerant, resistant to various inhibitors; isolated from mezcal
K. lactis GG799	YEPD and YEPG media at 30°C, 200 rpm for 96 hours	Haploid; a host for heterologous protein expression
K. lactis NRRL Y-1140	Complete YPD medium at 28°C	Haploid; known for β-galactosidase synthesis and lactose consumption
Kluyveromyces starmeri UFMG-CM-Y3682	YMA medium at 28°C	Isolated from cacti; helps study cactus-associated yeast communities
Kluyveromyces dobzhanskii CBS 2104	YPD medium at 28°C	Wild yeast, used for population genetics studies
Kluyveromyces wickerhamii UCD 54–210	10 L SSM at 25°C	Winery spoilage yeast, involved in toxin production studies
Kluyveromyces nonfermentans NRRL Y-27343	Grown at 22°C	Utilized in researching secondary metabolite gene clusters

Comparative functional assessments are needed despite the availability of Kluyveromyces genomic sequences. Budding is K. marxianus and K. lactis' main vegetative reproductive technique. In nature, the two yeasts might be haploid or diploid. Mating types a (MATa) and α (MATα) signify different genders. Meiosis allows diploid cells to generate four haploid spores in an ascus. K. marxianus mating areas have been established after comprehensive investigation of K. lactis. These areas contain HML, HMR, and MAT sites (19).

GG799 is a highly recognized commercial wild haploid strain of K. lactis. Moreover altering genes linked to the ability to switch mating types enables the manipulation of mating types to produce haploid organisms. Recent studies have suggested that the CRISPR/Cas9 system, without markers shows promise, in removing MATα3 or MATa1 in yeast effectively halting the switching of mating types. Furthermore promoting sporulation has been shown to result in the development of haploid strains. These techniques provide a way to select enduring haploid strains of Kluyveromyces (20).

1.2.2. Strong stress resistance and growth ability

Adaptability and Entericity are major features of cell function. K. Marxianus has the property of being better at bringing high temperatures produced at industry to ecology. With going for higher fermentation temperatures the expenditures connected to cooling, distillation and separation drop dramatically. It also increases its potential to enhance the competence of enzyme and reduce production costs, whereas at the same time decrease the chances of bacterial contamination. Unlike some yest strains that have their optimal temperature for growth K. Marxianus demonstrates strong traits of thermotolerance being able to well adapt to high temperatures and withstand even the temperatures of 50 °C (21).

It is known from studies that considerable amount of the cells will survive the heat shock damage if the trehalose is abundant in it (21, 22). We assessed the impact of temperatures on the genes of K. Marxianus via an observation and seen that expression of the genes associated with NADPH generation increased at its higher temperature. This reaction suggests that guarding NADPH neutralization might intensify cell antioxidant potential. Another thing in the icon of K. Marx (23), is the ability for her to endure another kind of heat.

These features are especially crucial when the body of the organism has to adapt to the higher environmental temperatures. Furthermore from the capacity of K. marxianus to resist high temperatures, the same unique microbe demonstrated excellent adaptability under acidic situations. In order to build this resource (library) we have conducted extensive work on mutants of RNA polymerase II TATA-binding protein (TBP). The aim was therefore to increase the strain's tolerance to acidity toward low pH (24).

The researchers in (8)proved that A had an impact on young people as high as 95%. 5- Alkaline environment as well contributed to the multiplication of the yeast, miliard of times more than the number of bacteria and other microorganism, making them the dominant organism in this basic fermentation. On the other hand no heavy metals harming the involved bacteria K. marxianus. Working for the adsorption of silver, copper, and cadmium in approximately 90%, 60%, and 65% degrees, respectively. KlYap8, a trans-regulator that prevents the detrimental effects of arsenite, antimonite, cadmium and hydrogen peroxide toxicity was revealed to be significant to the comprehension of the genomic analysis of stress responses through the study of (24). Hazardous chemicals are released during lignocellulosic substrate biomass biorefinery pretreatment. Chemicals may hinder microbial fermentation. Furfural, 5-hydroxymethylfurfural, phenols, and acetic acid. K. marxianus' tolerance to inhibitors at higher temperatures was studied using RNA-seq (26).

The expression of various genes related to redox balance, energy generation, metabolism, detoxification, and iron transport in K. marxianus was identified, indicating its tolerance to lignocellulosic inhibitors and enabling potential metabolic engineering for industrial fermentation using cellulosic biomass (27).

In recent times, due to its ability to withstand inhibitors, K. marxianus has emerged as a cell factory for the production of furan derivatives (28).

Compared to other species, K. marxianus has superior growth performance and stress tolerance. Table 3 compares Kluyveromyces strain development in different settings. Marxianus marxianus develops rapidly, peaking at 0.99 h−1 at 40 °C (29).

This makes it one of the most rapidly growing eukaryotes, as noted by (30). A maximum growth rate of 0.42 h−1 has been observed in K. lactis. Some researches claim K. marxianus fermentation may achieve 120 g/L cell biomass (29).

A maximum dry weight of 80 g/L has also been documented for K. lactis cells. In addition to K. fragilis, many species of Kluyveromyces have a notable rate of growth. Having been identified as having a maximal growth rate of 0.93 h−1, this specific species is generated from maize steep liquor and whey powder. (29).

Cell factories must typically attain a particular cell density in order to accomplish the intended rate of production. Exactly how long it takes cells to attain the production density is determined by their growth rate. With an increase in cell growth rate, the duration of the fermentation cycle decreases. When energy consumption is taken into account, the substantial advantages of Kluyveromyces for large-scale industrial applications are its swift growth rate and ample biomass (30).

Table 3. An analysis of the growth characteristics of several Kluyveromyces strains under distinct environmental conditions.

Strain	Temperature (°C)	Carbon Source	Fermentation Setup	Max Growth Rate (μmax, h^{-1})	Biomass Yield (g/L)
Kluyveromyces lactis	30	Whey	10 L fermentor, fed-batch	0.29 - 0.43	< 10
Kluyveromyces lactis CBS 2359	30	Glucose	300 mL flasks, batch	0.42	-
		Glucose	250 mL flasks, continuous	0.40	-
		Lactose	500 mL flasks, batch	0.37	-
		Sucrose	500 mL flasks, batch	0.40	-
Kluyveromyces lactis Y721	28	-	2 L fermentor, fed-batch	-	80
Kluyveromyces marxianus MTCC 1288	34	Crude whey	Flasks, batch	0.157	8.9
Kluyveromyces marxianus KMS2	37	Cheese whey	Shaker orbit cultivation	0.34	5.5

Kluyveromyces marxianus IMB4	40	Glucose	500 mL flasks	0.99	-
Kluyveromyces marxianus 6C17	37	Galactose	3 L fermentor, aerobic	0.37	3.82
Kluyveromyces marxianus CBS 6556	Multiple Temperatures	Various sugars	Various conditions	Varies (0.20 to 0.70)	Varies (3.82 to 103)
Kluyveromyces marxianus CCT 7735 (UFV-3)	37	Glucose	250 mL flasks	0.673	-
Kluyveromyces marxianus KM-PPV-VP2	30	Glucose	5 L fermentor, high cell density	-	120
Kluyveromyces fragilis	33	Whey powder	Fermentor, fed-batch	0.15 - 0.20	69.1
Kluyveromyces fragilis IMAT 1872	31	Whey powder	Flasks	0.93	-
Kluyveromyces sp. IIPE453	50	Xylose-rich bagasse hydrolysates	Fermentor	0.13	5.35

1.2.3. Wide spectrum of substrates

K. Marxianus is noted for its substrate versatility. K. Lactis and K. Marxianus can convert lactose into carbon (31). These two species naturally generate lactose-digesting enzymes that speed fermentation (14). Food processing waste is fermented with K. Marxianus to make ethanol. Marxianus' pectinase and inulinase enzymes make it a protein expression candidate (31). High inulinase yields enable Jerusalem artichoke fermentation for biorefinery. K. Marxianus' capacity to use xylose has helped it be used in cheap fermentation procedures using lignocellulosic biomass (14).

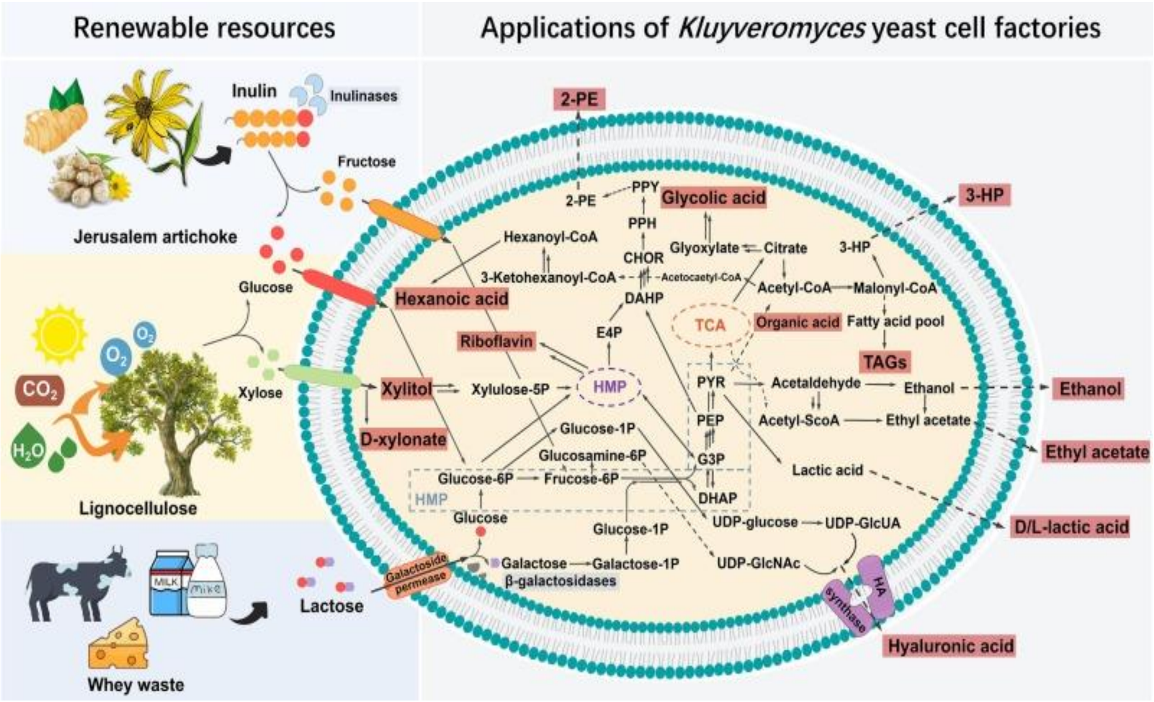


Fig. 3. Production of many compounds from renewable resources by the use of Kluyveromyces yeast cell factories

Agricultural waste

2.1. Overview

The global population has experienced a substantial surge in recent decades, reaching a staggering 7.9 billion by 2021, up from 3.7 billion in 1970. According to a study by (32), By 2050 and 2100, the global population is anticipated to increase to 9 billion and 11 billion, respectively. Hence, the task of guaranteeing food security in the future emerges as a substantial obstacle (33). A substantial increase in animal and cereal production has been implemented to meet the needs of a considerable population. This results in the generation of agricultural refuse (AWs). (34).

India generates 350-990 Mt of solid waste annually, with AW being the main source. India produces around 130 million tons of paddy straw yearly as a significant agricultural waste generator. Only 50% of this waste is used as feed and 50% is discarded. Rice residue (parali) burning in the northwest causes air pollution and public health risks (35).

Due to the improper dispersal of crop residue, greenhouse gases including carbon dioxide, nitrous oxide, and methane are produced. These substances possess the capacity to cause damage to both the ecological system and human life (35).

A multitude of agricultural byproducts have emerged as subjects of apprehension and jeopardise the environmentally soundness of agricultural systems. Agricultural byproducts, poultry byproducts, agricultural residues, agro-industrial byproducts, and aquaculture byproducts are all included (36).

A significant proportion of the AWs referenced are readily decomposable, and the byproducts of this process not only augment soil aeration and water retention but also give essential nutrients to plants. Supporting a clean, secure, and sustainable farm will cut greenhouse gas emissions and fossil fuel consumption while generating green markets and jobs by turning agricultural waste and byproducts into useful resources (37).

To distinguish environmental stresses from economic growth, it is therefore essential that the exploitation of agricultural residue be given top priority. This has to do with limiting the adverse impacts on soil, biodiversity, and global food security, as well as decreasing our dependence on basic resources (38).

Since cellulose accounts for 30-50% of agricultural biomass and has the potential for microbial metabolism, recent studies suggest that it can be used to make a variety of beneficial products (37).

These products can have significant applications in both households and commercial settings. There are various products that can be produced through research, such as compost, biocoal, and bioelectricity. In addition, it has the potential to provide new opportunities for young people from farming communities worldwide. Various studies have explored this topic (37, 39, 40).

Enhancing awareness among the general public and farming communities regarding the manifold benefits associated with biological and biotechnological approaches to agricultural waste management is of critical importance. The potential for increased income, reduced pollution of soil, air, and water, and enhanced human health are among these advantages. This would eradicate any preconceived notions and alarmist inclinations associated with the ambiguous information contained within (41).

Understanding a waste's origin, amount, and characteristics is essential to developing successful management or treatment techniques. There are four primary AW types: Agro-industrial, livestock, agricultural leftovers, and aquaculture waste (Fig. 4) (36).

Fig. 4. Various sources of agricultural waste.

2.2.1. Utilization of crop residue

Leaves, corn stovers, rice, wheat, oat, barley straws, and seed pods constitute agricultural waste. Recent estimates estimate worldwide agricultural residue output at 2802 Mt/y Sarkar et al., 2020. Only a small percentage of wheat, maize, and rice crop leftovers are utilized worldwide for bioethanol or animal feed. The leftover garbage is disposed of or burnt in the fields, which may harm health and agricultural sustainability (42).

It is noteworthy to mention that the annual rice straw production has a worldwide total of 731 Mt, with Asia accounting for 667.6 Mt of this total. Predictions indicate that by 2030, India's rice straw production will have increased to 221.8 Mt/y. On the contrary, it is estimated that wheat straw is produced at a rate of 1-3 tonnes per acre year, with an approximate annual worldwide output of 354.34 million tonnes. Globally, corn is a key commodity in the manufacture of lignocellulosic bioethanol (43).

According to study conducted by (44), annual production of maize stover (containing stalks, leaves, cobs, and husks) is estimated at 128.02 Mt/y. Furthermore, it has been observed that the yearly yield of corn stover amounts to 4.0 tonnes per acre. **2.2.2 Livestock waste**

Wastewater, solid manure, and liquid manure are livestock waste. Wastewater includes agricultural water, liquid manure, disinfectants, bedding, and silage juices. Solid manure, or farmyard manure, is another frequent animal waste. Last, liquid manure is urine. The European agriculture industry produces 1500 Mt/y of animal manure. These waste products include 1284 million tons of cow dung/excrement and 295 million tons of pig manure (45).

Air and water pollution from untreated manure may occur. Pathogens and nutrient-rich trash may pollute surface water. Manure releases carbon dioxide (18%) and methane (57%), adding to the greenhouse effect (45).

2.2.3. Agro-industrial waste

Food processors produce agro-industrial waste, an agricultural byproduct. These goods come from sugar, rice, fruit, vegetable, and oil companies. They include deoiled cakes, molasses and bagasse, rice husk, fruit and vegetable skins and pomace (orange, mango, apple, and litchi), starch residue, eggshells, poultry and farm animal skin, and meat (3).

Sugarcane bagasse is a significant byproduct that is left behind once the sugarcane juice has been extracted. Each year, a staggering amount of sugarcane bagasse is produced globally, with projections indicating that this number will continue to rise in the coming years. The production of palm oil is a significant contribution from the world's leading edible oil industries. As a result, a substantial amount of palm kernel-cake is generated from fresh fruit bunches, totaling 35.19 million tonnes from 85.84 million tonnes (3).

Horticulture-dependent food industries produce a substantial volume of byproducts, including pomace, fruit rind, and seeds. During the production and refining of a variety of goods, including cider, citrus juice, preserves, jellies, condiments, ketchups, and relishes, these residues are generated. Consider the following example: apples: An astounding 767,75,294 MT of apples are produced annually on a global scale, with apple pomace comprising between 25 and 30 percent of the total biomass (46)

Now, let's consider the scenario with different types of fruits. Food processing industries also package intact fruits and vegetables for sale in wholesale and retail markets. The food processing industries in China, US, India, and other countries produce large amounts of organic waste through their packing, processing, storing, and distribution activities (46).

2.2.4. Aquaculture waste management

The rise in global human population has also led to a surge in the need for fish (47). Aquaculture has undergone significant advancements due to improved cultivation techniques and is anticipated to supply approximately two-thirds of the fish needed for global consumption by 2030. As a result, this sector is experiencing significant growth within the agricultural industry. Aquaculture comprises the processes of reproducing, rearing, and harvesting aquatic vegetation, crustaceans, and fisheries both freshwater and marine, in addition to endangered species conservation initiatives. Significant expansion is occurring in this sector, which is an indispensable contributor to the global food supply (47).

It is worth mentioning that from 1990 to 2016, there was a significant increase in global aquaculture production. This growth was particularly notable from 2000 to 2016, with an average annual increase of 5.8 percent (48).

Aquaculture supports a "blue revolution." by ensuring food security and meeting the nutritional demands of a big population. Water pollution and feed waste grow with aquaculture development. This harms the ecology and aquaculture sustainability (49). The quantity of feed needed is influenced by the culture type, as stated by (50).

Studies have shown that feed has emerged as a significant source of waste in aquaculture (50). According to(50), the main contributors to solid waste in aquaculture are unused feed and fish faeces. Research has shown that solid wastes can have a detrimental impact on fish populations. They can obstruct the fish's gills, resulting in fatality. It is crucial to remove them as soon as possible (50).

Another important aspect to consider is the presence of dissolved wastes, which primarily consist of metabolic byproducts or leftover food. The feed contains significant amounts of nitrogen and phosphorus, contributing to the high levels of waste. P and N play a crucial role in the protein component of the fish, as highlighted by (51). Fishes have the ability to retain significant amounts of nitrogen and phosphorus. They struggle to make use of a significant amount of P and N, resulting in over 50% of these nutrients remaining in the culture water. This, unfortunately, leads to water pollution and the associated environmental impacts (52).

Elevated turbidity and facilitated algal and microbial proliferation may result from the presence of these nutrients, hence detrimentally affecting the water's suitability for fish. Potentially harmful to aquatic creatures and a contributor to eutrophication, the release of this water into other bodies of water might result in pollution. Nitrite and nitrate may be produced from the ammonia that is discharged into the culture water. Unionized and ionised forms of ammonia are distinct states, denoted by NH3 and NH4+, respectively. Temperature and pH of the water determine the proportions of the two substances as they are balanced in it. As opposed to ionised particles, the condition with unionised employees is more damaging, according to (53).

Despite the fact that nitrite at a concentration of 200 mg/L may be detrimental to the culture, it is oxidised to produce nitrates, which are harmless. Phosphates and nitrates, on the other hand, may hasten the deterioration of an aquatic environment. The effect of phosphates on eutrophication is contingent upon several elements, including concentration, release frequency, and the dimensions of the waterbody, with phosphates being discharged in the form of particles in faeces. As a consequence, much emphasis has been placed on aquaculture as a strategy to mitigate water pollution and improve the survival prospects of marine organisms (54).

2.3. Management of agricultural wastes

Agricultural waste is a substantial quantity that is produced on a yearly or biannual basis in each nation and state around the globe. Hence, in order to save the environment and advance sustainable agriculture, it is essential to proactively manage, repurpose, or transform it into goods that enhance value. Table 2 outlines the advantages and disadvantages of the AW management options (36).

Table 4. Comparative assessment of agricultural wastes management strategies (36).

Waste Management Strategy	Agricultural Residues	Pros	Cons
Animal Feed	Vegetables, corn, sugarcane, grain, sorghum, soybeans	Nutrient source for livestock, reduces waste	Potential contamination with toxins from molds, fungi, and chemicals like pesticides and herbicides
Roof Thatching	Straw, palm leaves, water reeds	Long-lasting, insulating, environmentally friendly, aesthetically pleasing	Labor-intensive installation, requires yearly inspections and maintenance, risk of fire and insect infestation
Surface Mulching	Bark chips, grass clippings, various straws, etc.	Moderates soil temperature, reduces erosion, enriches soil nutrients	Can hinder soil oxygenation and drainage, may contain weed seeds, fosters insect and pest habitats
Composting	Leaves, grass clippings, food scraps, plant debris	Produces organic fertilizers, mitigates odors and weeds, environmentally sound	Requires costly setup and equipment, process duration can be long, may generate dust and odors

Fertilizer Preparation	Straw, corn stalks	Enhances soil nutrient retention and structure	Organic fertilizers may lack certain nutrients
Direct Combustion	Woodchips, sawdust, bark, hog fuel, black liquor, etc	Utilized for heating, cooking, and power generation	Higher energy production costs
Pyrolysis	Various agricultural residues	Degrades pathogens, produces char and low-grade fuel, recovers energy	Complex and costly process, requires further treatment of flue gases, ashes may contain heavy metals
Biochar	Agricultural and agro-industrial wastes	Absorbs pollutants, high energy content	Crop response to biochar varies significantly
Bio Coal	Various agricultural residues	Chemical-free, efficient burning, easy storage	High operational costs, potential combustion issues like smoke, susceptible to moisture
Fibres	Various plant-based residues	Alternative or supplement to wood in furniture manufacturing	Time-consuming process
Bio-bricks	Various agricultural residues	Eco-friendly building material, suitable for affordable housing	Lower strength than traditional bricks, not suitable for load-bearing structures
Paper & Pulp Industry	Various plant-based fibers	Environmentally friendlier than wood, prevents deforestation	Economic challenges related to harvesting, transportation, storage, and production
Mushroom Cultivation	Various agricultural residues	Cost-effective, addresses environmental issues and hunger	Limited local market promotion opportunities
Adsorbents for Heavy Metals	Various plant-based residues	Effective low-cost solution for heavy metal removal	Requires further research
Electricity Generation	Various agricultural residues	Renewable, reliable, reduces waste	High setup and space costs
Organic Acid Preparation	Various agricultural residues	Potentially reduces production costs	Still at the lab-scale with limited real-world data
Industrial Enzyme Preparation	Various plant-based meals and brans	Economical source of carbohydrates for industrial use	Lengthy process
Biofuel Production	Various crops and residues	Renewable, reduces dependency on fossil fuels, minimizes waste	High costs associated with biomass energy plant installation

Flavor Compound Production from Agricultural Waste

3.1. Flavor compounds

Flavor chemicals are essential to food flavor and scent. These chemicals are generated via amino carbonyl reactions, Maillard reactions, and amino acid-peptide interactions, especially sulfur compounds. Retronasal aromas and food matrix odor-active chemicals help distinguish tastes (55). Reaction components, environment, and circumstances alter taste-protein interactions and flavor delivery. They use mass spectrometry to study how taste chemicals interact with dietary proteins (56).

According to the flavor coupling theory (57), foods and beverages are considered to be complementary when they contain essential flavor compounds that enhance sensory attributes and appeal to consumers. During food processing, such as dehydrating, the flavor profile of the final product is affected by the retention of flavor compounds.

Covalent adduct formation between taste chemicals and proteins affects food flavor throughout heat treatments. Plant-based meat replacements mimic typical meat products' flavors using descriptive sensory qualities and volatile flavor molecules (58).

By using gas chromatography-mass spectrometry, important fragrance ingredients, such as minty chemicals, may be identified, helping to understand food taste profiles (56). Volatile compounds, flavonoids, and phenols are constituents of plants that impart flavor and nutritional value. Additionally, secondary metabolites such as amino acids, organic acids, and esters affect the aroma and flavor of fruits such as mangoes (59).

Pertaining to the realm of food and beverage manufacturing, particular compounds such as nitrogen and sulfur compounds play a substantial role in the development of unique fragrances and flavors, as evidenced by the observations made in coffee and sesame seeds. Volatile compounds, which have been detected in a variety of food items including honey and mutton sausage, serve as indicators of their botanical provenance and contribute to the distinctive flavors of these products. In addition, during the maturation process of whisky production, significant flavor compounds emerge that function as indicators of the whisky's age (60).

3.1.1. Flavor compound classification:

Different criteria classify flavor chemicals. A thorough taste compound classification (61):

1. Chemical Structure: a) VOCs: Low molecular weight and boiling point characterize VOCs, aliphatic and aromatic chemicals. VOCs come from solvents, dry cleaners, degreasers, paints, chemical intermediates, and industrial items. They are combustion and drinking water chlorination byproducts. VOCs may also be produced while microwaving. Some VOCs from commercial packaging are allowed as indirect food additives. This category comprises volatile chemicals that give meals and drinks their scent. Aldehydes, ketones, alcohols, esters, terpenes, lactones, and sulfur compounds are VOC subclasses (61).

b) Non-Volatile Organic substances: These substances enhance taste perception and balance. Non-volatile compounds include sugars, amino acids, peptides, proteins, organic acids, and others (61).

3.1.2. Causes for food volatiles

Recipe components, cooking techniques, storage conditions, and microbiological activity may cause volatiles in food. Some volatile food components are undesirable. Off-flavors and off-odors in meat, dairy, and vegetable products may indicate spoiling. High-heat cooking and starchy food storage may produce toxic acrylamide, which can compromise food safety. For product quality, safety, and consistency, volatiles in food must be monitored (62).

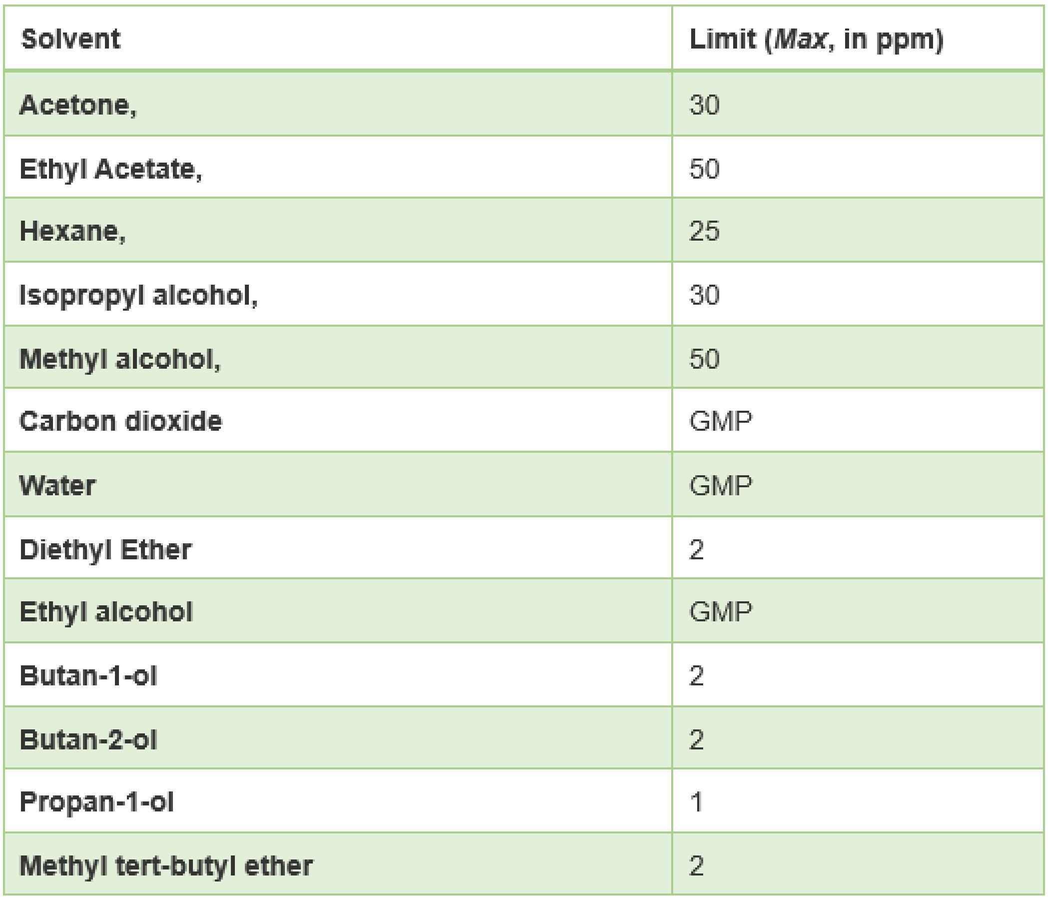
3.1.3. VOCs (volatile organic compounds) are present in food and beverage processing (63):

1. VOC-emitting tastes and perfumes are found in many food and beverage goods. Ethanol, acetone, and acetaldehyde may be found in natural and manufactured flavorings for beverages, snacks, and baked products.
2. Packaging materials: Food and beverage packaging may produce VOCs, especially during manufacture. Toluene, benzene, and xylene may be released via packaging adhesives, coatings, and inks.
3. To assure food safety and quality, the food and beverage sector uses numerous cleaning and sanitation procedures. Cleaning products and disinfectants using solvents or other volatile substances may emit VOCs.
4. Beer, wine, and cheese fermentation and brewing create VOCs. These chemicals may influence product tastes and smells.
5. Processing and cooking: Some food and beverage cooking and processing may create VOCs. PAHs, a carcinogenic VOC, may be produced by grilling or frying meat.

3.1.4. Regulations and standards

FSSAI lists various food-grade solvents for spice oleoresin extraction and associated residual limits in table (5) (64):

Table 5. FSSAI lists various food-grade solvents for spice oleoresin extraction and associated residual limits .



Solvent	Limit (*Max*, in ppm)
Acetone,	30
Ethyl Acetate,	50
Hexane,	25
Isopropyl alcohol,	30
Methyl alcohol,	50
Carbon dioxide	GMP
Water	GMP
Diethyl Ether	2
Ethyl alcohol	GMP
Butan-1-ol	2
Butan-2-ol	2
Propan-1-ol	1
Methyl tert-butyl ether	2

3.2. The importance of flavor compounds:

Food taste and sensory perception depend on flavor molecules. These chemicals affect taste release and perception with main food additives. Foods with proteins, polysaccharides, lipids, and salt modify aroma compound volatility and taste perception. Flavor contribution includes many interactions, making it difficult to identify flavor-affecting substances (65). Fluid food flavor-active ingredients and their hydrophobicity and solubility (ChemAxon) (Figure 5) (66).

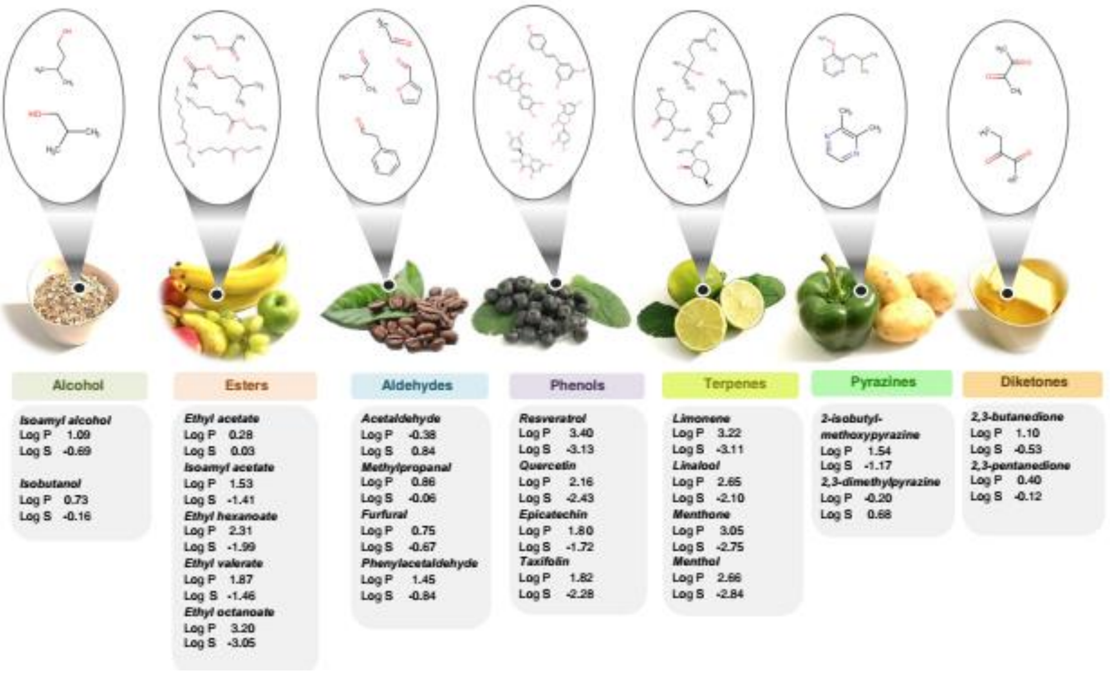


Fig.5. Main flavor-active components in liquid foods and their physical properties (Hydrophobicity and solubility) (ChemAxon) (66).

Food flavour evaluation relies on volatile molecules. Protein-flavor component binding depends on chemical structure, food matrix composition, and processing condition. Chemoreceptors activated by taste molecules cause conscious food flavor perception (67).

Intramuscular fat content affects beef's sensory qualities and taste, enhancing certain flavors and decreasing others. taste molecules are solubilized in meat lipids, making them essential to taste production. Key chemicals in tomatoes and rice enhance taste perception, according to research (68). Studies on how drying processes affect taste components and flavor characterisation of particular foods have demonstrated that processing methods affect flavor profiles (69).

Olfaction is crucial to taste perception. Game meat's sensory qualities and bioactive chemicals' effects on flavor precursors show how complicated taste creation is in diverse foods.In light of childhood obesity, understanding how taste preferences and sensitivity affect flavor perception is vital. Volatile fragrance molecules, organic acids, and soluble solids in fresh produce like cucumbers have also been studied, showing that chemical composition affects refreshing perception (70)

3.3. The nutritional importance of flavor compounds

Flavor molecules are crucial to food flavor and smell. These chemicals have fruity, bitter, fatty, and flowery tastes. Flavor compounds and food elements must combine to provide tasty, healthy meals. Ionic strength, pH, and temperature affect taste-protein interactions and food matrix flavor release (71).

Flavor metabolites change during fermentation, affecting microbial populations. As fermentation continues, undesirable chemicals decrease and good ones rise, improving product quality. Volatile molecules with an olfactory activity value (OAV) > 1 are considered crucial for taste in Pu-erh tea (72)

The flavor profile of fermented foods like Chinese Dongbei suancai is enhanced by short-chain acids, alcohols, and esters. Steaming and microwave cooking boost important taste molecules such aldehydes, which provide food fruity, almond, fat, and grassy flavours (72).

Changes in physicochemical parameters affect both bacteria and taste molecules during fermentation. Flavor molecules typically have nutritional advantages, therefore increasing terroir may also affect nutritional value (73)

Tetramethylpyrazine in Chinese baijiu adds fragrance and nutrition. Understanding the complex link between microbes and taste components may help discover flavor-forming bacteria and regulate fermented food flavor (74).

The use of Kluyveromyces in the production of flavor compounds from some agricultural wastes

4.1. Investigating the production of flavor compounds through the metabolic processes of Kluyveromyces

Investigating Kluyveromyces yeast strains for taste compound synthesis signifies an auspicious and pioneering domain within the realms of biotechnology and food. Microorganisms known for their versatility, specifically strains such as Kluyveromyces lactis and Kluyveromyces marxianus, have demonstrated considerable promise in the field of bioconversion. By converting a wide range of substrates into flavour compounds of high value, these microorganisms improve the sensory attributes of food and beverages Table 6 (75).

Table 6. Overview of typical flavor compounds of Kluyveromyces sp.

Strains	Bio-products	Characteristics of regulate strategies	Type	Values	References
***K. marxianus* CBS1555**	Ethanol	Fed-batch fermentation with simultaneous saccharification at 37°C	Fermentation	70.20 g/L	(76)
***K. marxianus* ATCC 36907**		72-hour SSF of sunflower grain at 150 rpm and 38°C, with variable enzyme concentrations	Fermentation	27.88 g/L	(77)
		SSF using pretreated cashew apple bagasse	Fermentation	18.00 g/L	(78)
***K. marxianus* YZJ088**		Engineering xylose assimilation for co-fermentation of glucose and xylose	Genetic Modification	51.43 g/L	(79)
***K. marxianus* DBTIOC-35**		SSF with pretreated wheat straw	,Fermentation	66.20 g/L	(80)
***K. marxianus* DMKU3–1042**		Engineered TATA-binding protein to enhance ethanol tolerance	’Genetic Modification	57.29 g/L	(81)
***K. marxianus* MTCC 1389**		SSF at 41°C with set woody stem Prosopis juliflora	’Fermentation	21.45 g/L	(82)
***K. marxianus* K21**		Enhanced ethanol production from sugarcane bagasse treated with ambient cold plasma	Fermentation	5.20 g/L	(83)
K. marxianus		Enzymatic hydrolysis of apple pomace with soluble soy protein addition	Fermentation	29.50 g/L	(84)
***K. marxianus* JKH5 C60**		Utilizing multi-stress tolerant yeast developed via adaptive laboratory evolution	Genetic Modification	54.80 g/L	(85)
K. marxianus* CBS 6556Δ*ura3	Ethyl acetate	Regulation of core carbon flow for ethyl acetate biosynthesis using multiplexed CRISPRi	Genetic Modification	–	(86)
***K. marxianus* NBRC1777**	2-Phenylethanol	Overexpression of phenylpyruvate decarboxylase	Genetic Modification	–	(87)
***K. marxianus* CCT 7735**		Optimized fermentation conditions at 30°C with glucose and L-phenylalanine concentrations	Fermentation	3.44 g/L	(88)
***K. marxianus* KmASR.129**		Feedback-resistant phenylalanine biosynthesis, enhanced precursor availability	Genetic Modification	0.85 g/L	(89)
***K. marxianus* CBS 6556**		Refactoring of the shikimate pathway	Genetic Modification	1.94 g/L	(89)
K. marxianus		Enhanced fermentation conditions using sweet whey	Fermentation	1.2 g/L	(90)
K. marxianus* ATCC 17,555Δ*ura3	Hexanoic acid	Development of hexanoic acid pathways integrating multiple gene permutations	Genetic Modification	0.15 g/L	(91)
***K. marxianus* K326**	Γaminobutyric acid	72-hour fermentation at 28°C	Fermentation	7.78 mg/L	(92)

4.2. Investigating the production of flavor compounds from rice bran through the metabolic processes of Kluyveromyces marxianus and Debaryomyces hansenii yeasts.

A new work of Guneser et al. « 2022 (93) » Fish' Fermentation with Kluyveromyces marxianus and Debaryomyces hansenii has been reported to be responsible of taste chemicals. Yeast cells' growth rates and doubling times were measured by us and the results reported. GC-O, GC-MS and Spectrum™ are employed in order to study the sensory impression of the synthetic phosphate compounds. The rate of specific growth (μ) and doubling time (td) as found for K. marxianus were at 0. 16/h and 4. 21h, respectively. To differentiate from D. hanseni, which is favorable to grow at zero, the other two strains do not necessarily have to cover the same conditions. In the meantime, the virus will wreak havoc by multiplying itself by 13 times and sometime rambling at 5. 33 hours. The strain K. marxianus and as well as D. hansenii were found to generate more alcohols and ethyl acetates respectively, from the rice bran. Research points to 216 g of K. marxianus that could be manufactured. Ethyl phenyl acetate, µg/kg 08, 827. The data analysis also indicates that the isoamyl alcohol concentration is 27 µg/kg, which is the highest among the coffee brewed with other label. Phenyl alcohol (24-hour bulk fermentation is created by 77 µg/kg. 4.8 g/kg of isovaleric acid was produced by K. marxianus in 96-hours bulk fermentation.

Managing Water Resources in Arid and Semi-Arid Regions There was 135 yild of D. hansenii-fermented rice bran after 24 hours. Come 77 µg/l phenyl ethyl acetate and 415 mg/kg. 64 µg/kg isoamyl alcohol. Traditional fermented rice bran revealed the traits of fermented rice bran which include that it tastes like fermented grains. During fermentation, the fragrance of bran rose too. There are phenyl ethyl alcohol, guaiacol, acetate, and isoamyl acetate that stand for the rice bran fermented sourish character that is described as rose taste. This research showed that yeast fermentation process variables generates natural tastes which are customer-friendly and hence, enhances the value of rice bran.

Coming to tomato and pepper pomace, we have earlier convey the behaviours of their aroma production by K. marxianus and D. hansenii. Bioengineering uses the process of microbial and plants metabolism as a flavoring method. Tomato and red pepper pomace were the two sources of K. marxianus and D. hansenii, the two species of microorganisms, that were used for the production of bioflavor. Tomato plant remains, especially the K. marxianus and D. hansenii strains, exhibited the highest growth rates. 081/h and 0. 177/h. A lot of yeasts for the flavors, differently. The production of isovaleric acid, phenylethyl alcohol, isoamyl acetate, and alcohol is used by both yeasts in their metabolism. Using k. marxianus, pomaces of tomato and pepper were fermented and evaluated as "new tarhana" and "rose". E. Hansenius-fermented tomato pomace had taste of pea and pepper pomace like storage/yeast and fermented vegetable. K. Marxianus and D. Hansenii olea palamatia Lactobacillus and Bifidobacteria pomace fragrance. They discovered that bulgur pocketed wet smell was coming from probiotik D.Hansenii fermented tomato pomace. In 2021 Kılmanoğlu et al carried out an investigation (Kılmanoğlu et al., 2021) and 8 days after food preparation, there was a rosy scent in the tomato pomace. 5-hour brewery job by K. Marxianus.

Results and Discussion

Table 7. The biotechnological utilization of three strains of Kluyveromyces for flavor compound production from agricultural wastes.

Strain	**Substrate Used**	**Flavor Compounds Produced**	**Biotechnological Process**	**Advantages**	**Source**
Kluyveromyces marxianus	Agricultural wastes	Ethyl acetate, 2-phenylethanol	Cell factory applications using	Thermotolerance, rapid growth, wide	(96)

Strain	Substrate Used	Flavor Compounds Produced	Biotechnological Process	Advantages	Source
			synthetic biology and fermentation optimization	range of volatile molecule production	
Kluyveromyces marxianus	Lignocellulosic wastes	Variety of volatile flavor compounds	Utilization of synthetic biology tools and CRISPR-Cas9 for pathway engineering	Production flexibility using various carbon sources, resistance to inhibitors	(97)
Kluyveromyces marxianus	Whey	Beta-galactosidase, oligonucleotides	Fermentation and fed-batch processes	Utilizes dairy waste, produces high-value bioingredients, reduces pollution	(98)
Kluyveromyces lactis	Cheese curd	Volatile sulphur compounds (VSCs)	Growth in cheese medium, degradation of l-methionine	Produces a wide variety of VSCs, enhances cheese flavor	(99)
Kluyveromyces lactis	Guava waste	Volatile aroma compounds (fruity, floral)	Submerged fermentation	Utilizes waste, produces high-value	(100)
Kluyveromyces fragilis	Whey permeate	Glycerol	Anaerobic growth conditions using whey permeate as substrate	High glycerol production, utilizes cheese whey lactose	(101)
Kluyveromyces fragilis	Cheese whey	Beta-D-galactosidase	Growth in cheese whey, optimization with ammonium sulfate and yeast extract	High enzyme activity, enhances lactose hydrolysis	(102)

This research shows that, Kluyveromyces marxianus, among others has a great potential for economical and eco-friendly natural flavor processing. This is done by modifying biotechnological methods to convert useless farm remnants into profitable substances hence acting as an option to artificial flavors that might be harmful both to people and the environment in general.

Conclusion

The utilization of Kluyveromyces strains in the biotechnological synthesis of flavor compounds from agricultural and dairy residues not only promotes waste minimization and environmental preservation but also contributes to the financial feasibility of the food and beverage sectors. Each strain—K. marxianus, K. lactis, and K. fragilis—offers specific benefits to the manufacturing procedures, ranging from elevated product yields to the capacity to operate under diverse environmental circumstances. Subsequent investigations and advancements are anticipated to concentrate on enhancing the effectiveness and expandability of these procedures, steered by progress in genetic manipulation, process enhancement, and enhanced comprehension of yeast metabolism. The ongoing exploration of these yeasts shows potential for the creation of innovative, sustainable approaches for flavor synthesis that also conform to consumer inclinations towards natural products.

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