An Approach on the Functional Properties of Mulberry Leaves Associated with Yeast (S*accharomyces cerevisiae)* for Silkworm *Bombyx mori* Feeding

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Abstract

This paper explores the characteristics and associative impact of the compounds from mulberry leaves and yeast (*S. cerevisae*). Nutritional characteristics of the mulberry leaves, yeast properties and metabolism, as well as the effects on silkworms, were described. The online English databases used were MDPI, PubMed, Research Gate, Google Scholar, and Elsevier. The nutrition and health of silkworms *B. mori* depend on protein and amino acids, fats, vitamins, and minerals, all abundant in mulberry leaves. Furthermore, mulberry leaves contain bioactive phytochemicals offering health benefits. Many dietary supplements were investigated for their potential to enhance nutritional, technical, and health benefits, even though mulberry leaves are considered a complete feed for *B. mori* silkworm. Yeast is a good source of proteins, amino acids, fats, carbohydrates, minerals, and vitamins that can contribute to the fortification of mulberry leave characteristics. *S. cerevisiae* species also produce several secondary metabolites: polyketides, phenolics, alkaloids, and flavonoids. Numerous studies have demonstrated the positive effects of yeast dietary addition on economic indicators and health status in silkworm fifth instar. Yeast can act as a probiotic, release digestive enzymes and influence gut microbiota. Alterations in the gut microbiota improve immunological resistance and nutrition metabolism, ameliorating silkworm performances.

Keywords: insects, mulberry leaves, probiotics, silkworm, yeast.

1. Introduction

One of the most beneficial monophagous insects fed mulberry leaves (*Morus spp*.) is the silkworm *Bombyx mori* L. (*B. mori*) [1]. The capacity of the silkworm to convert protein from mulberry leaves into silk proteins is one of its most significant traits. The starting point of silkworm rearing is mulberry trees (Morus genus, Moraceae family), the predominant species *Morus alba*, widely distributed throughout both tropical and temperate regions [2,3].

Natural products are currently receiving more attention to handle various health issues. Considerable studies have been conducted on the relationship between nutrition and health, while pharmaceutical, nutraceutical, and functional products have been developed [2].

The mulberry leaves are a great source of vitamins, minerals, and amino acids, all required for silkworms *B. mori* nutrition and health.

Mulberry leaves provide several health advantages [4]. Current insights on the functional benefits have shown that the bioactive phytochemicals found in mulberry leaves exhibit a broad range of biochemical properties, such as antioxidant, hypolipidemic, anti-diabetic, anti-obesity, anti-hypertensive, and anti-atherosclerotic.

The potential and applicability of biotechnology in boosting sericulture productivity have been clearly highlighted recently in numerous scientific papers. It is an effective instrument for the silk industry's

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and sericulture's long-term growth and development [5].
A beneficial modern technology that can raise the economic value of cocoons is the fortification of mulberry leaves with additional nutrients [6]. Over years, probiotics have been investigated as possible dietary supplements. Probiotics are live, non-pathogenic bacteria that improve health by enhancing the balance of gut microflora and by increasing cellular growth and development when are taken in sufficient quantities [7,8].
Probiotics have the potential to enhance nutritional value by generating vitamins, detoxifying, and breaking down digestible substances [8]. Furthermore, Amala et al. [9] cited by Taha et al., [7], highlighted that probiotics should enhance silkworm immunity rather than manage the disease conditions.
Several studies were conducted on the effects of probiotics (*Saccharomyces cerevisiae* and effective bacteria) associated with mulberry leaves to alter the economic parameters of *B. mori* larvae in their fifth instar [3,7,10]. Thus, dietary supplements have been used to investigate the effects on economic indices, the capacity to resist diseases, and the molecular structure of a protein in silkworms. In the silkworm, *Saccharomyces cerevisiae* (*S. cerevisiae*) functions, for example, as an immunomodulating agent [3].
It was proved that microorganisms can boost the bioactivity of the bioactive compounds [4].
This study emphasizes the characteristics and associative effects of the bioactive substances from mulberry leaves and yeast.

2. Materials and methods

This review covers knowledge regarding the nutritional and functional characteristics of the mulberry leaves and yeast as well as their impact on the silkworms *B. mori*. The online database publications were used. The English scientific literature was taken into consideration, such as MDPI, PubMed, Research Gate, Google Scholar, and Elsevier. When browsing, the following keywords were used: silkworm; mulberry leave; yeast (*S. cerevisiae*); larvae; bioactive compounds; probiotics, and combination of words such as: yeast metabolism, mulberry leave nutritional characteristics; probiotics to silkworm *B. mori*; yeast functional and nutritional characteristics; silkworm health.

3. Mulberry leaves characteristics

The Moraceae family includes at least 100 recognized varieties, more than 64 species, and subspecies. The Latin term "*mora*" (Morus) means "delay," most likely due to the buds' sluggish development. Mulberries were first cultivated by the ancient Greeks and Romans. Since 220 A.H., Emperor Elagabal was wearing a silk coat, and later, in the 1500s, religious leaders in England had silk robes [11].
The leaves are crucial from an ecological perspective and as a single feed for silkworms *B. mori*. About twenty volatile chemicals are responsible for the silkworms' attraction to mulberry leaf consumption. The larva has receptors for the chemicals Ɓ-Ɣ hexenol and α-Ɓ hexenal, found in the essential oil of mulberry leaves. The antennae of silkworm larvae have twenty active olfactory receptors. Among these receptors, *cis*-jasmone is believed to be the most effective and attractive volatile, although it is present in leaves in trace amounts (0.3–300 ng). Insects move toward the odour source when an olfactive receptor is stimulated to become active. When administered in amounts between 30-300 ng, but not in tiny amounts, citral also attracts silkworms. At 30 ng or 3 ng, linalool, hexenal, hexyl acetate, and acetate 2-hexenyl showed an appealing activity [12,13].
Mulberry trees are cultivated in various climatic regions, highly adaptable and resistant to harsh environments [4,14-15]. Leaf quality and quantity have a significant impact on the quality and quantitative parameters of the cocoons. A higher nutritional value of leaves not only improves the products' quality but also strengthens the resistance to illness of insects [16]. The quality of mulberry leaves used to feed silkworms represents a critical factor contributing to about 38% of the total success of cocoon development [17].
Nonetheless, the leaves contain high amounts of the polymer building blocks of lignocellulosic compounds, namely 14–28% lignin and 12–36% cellulose [18]. This can reduce silkworms' capability to efficiently utilize the feed by physically shielding the protein and carbohydrates in the leaves' cell content from enzymatic hydrolysis [17].

Proteins, carbohydrates, vitamins, sterols, and minerals are the primary nutrients found in mulberry trees [13,16,19]. Data collected from the online literature database revealed the following chemical composition of mulberry leaves (Tables 1 to 4).

Table 1. Proximate composition of mulberry leaves (% as DM bases)

Specifications*	Mean	Minimum	Maximum	SEM
Dry matter, DM	28.01	17.6	46.3	4.90
Protein	23.56	11.8	39.6	4.77
Lipids	4.93	1.34	13.18	2.37
Carbohydrates	27.84	1.77	56.42	21.43
Fibre	13.35	5.40	30.80	4.63
Cellulose	13.45	12.1	14.8	1.91
Ash	13.22	4.50	22.36	3.43
NDF	32.03	19.4	49.7	6.76
ADF	22.85	16.5	31.8	4.84

*Dry matter, DM; neutral detergent fiber, NDF; acid detergent fiber, ADF. References: Chundang, [17], Sanchez-Salcedo [20], Thaipitakwong et al., [21], Iqbal, [22], Adeduntan and Oyerinde [23], Muzamil [24], Alidee, [25], Kumar et al, [26], Chang, [27], Imran et al., [28], Doliş et al., [29], Dolisş et al., [30,31], Vu et al., [32], Srivastava et al., [33], Kandylis, [34], Yu, et al., [35], Astuti et al., [36], Ekastuti et al. citat de Astuti [36], Kang et al., [37], Sahoo et al., [38], Ustundag et al., [39].

Table 2. Amino acids content in mulberry leaves (%)

Specifications*	Mean	Minimum	Maximum	SEM
Essential amino acids				
Lysine	2.09	1.0	5.0	0.35
Methionine	0.54	0.21	1.89	0.12
Threonine	1.54	0.77	4.0	0.25
Arginine	1.27	0.88	5.0	0.07
Histidine	0.89	0.35	3.56	0.21
Valine	1.83	0.85	5.0	0.32
Phenylalanine	1.80	0.91	5.0	0.30
Isoleucine	1.54	0.74	5.0	0.29
Leucine	2.45	1.45	7.0	0.34
Tryptophan	0.36	0.26	0.54	0.02
Total essential amino acids	**14.31**			
Non-essential amino acids				
Alanine	1.46	1.09	1.58	0.04
Glycine	2.0	0.86	6.0	0.40
Proline	0.75	0.33	1.31	0.10
Serine	1.42	0.66	4.0	0.25
Tyrosine	0.80	0.62	0.89	0.02
Cysteine	0.20	0.11	0.30	0.02
Aspartic acid	2.57	1.50	5.0	0.27
Glutamic acid	3.23	1.0	11.0	0.59
Total non-essential amino acids	**12.43**			

References: Machii et al., [40], Machii & Katagiri [41], Yao et al., [42], Olteanu et al., [43], Wang et al., [44], Al-Kirshi [45] Astuti et al., [36].

It is relevant that the online database presents a wide variety of values reported and the significant variability. However, data processed by us show the higher nutritional value of mulberry leaves, rich content in essential nutrients such as proteins (value ranged between 11.8 to 39,6 % on DM bases), and their amino acids (leucine and lysine being the most predominant essential amino acids, 2.45 and 2.09%), an excellent composition in PUFA, predominant being n-3 PUFA known for their health positive implication (the higher level noted for C18:3n-3), and a valuable mineral and vitamins content as well.

Table 3. Mineral composition of mulberry leaves (g x Kg^{-1} DM)

Specifications*	Mean	Minimum	Maximum	SEM
Macro elements (g x 100 g^{-1})				
Calcium	2.20	0.80	5.0	0.16
Potassium	2.03	0.30	3.90	0.26
Phosphorus	0.41	0.10	2.20	0.12
Sodium	0.01	0.01	0.01	0
Magnesium	0.77	0.50	1.40	0.12
Sulfur	0.21	0.20	0.30	0.01
Microelements (mg x Kg^{-1})				
Iron	159.56	119.30	241.80	17.17
Zinc	30.97	23.9	39.5	2.14
Manganese	57.77	35.80	90.50	6.94
Copper	5.21	4.20	5.90	0.28
Molybdenum	1.47	0.80	2.30	0.21
Boron	436.17	253.50	825.30	77.98
Nickel	2.81	1.70	5.40	0.49
Lead	0.49	0.30	0.80	0.07
Lithium	6.77	1.90	17.20	2.06
Titanium	6.97	5.40	10.80	0.73

References: Sanchez-Salcedo et al., [20], Ustundag et al., [39], Thaipitakwong et al.,[21], Adeduntan, [46], Bamikole et al., [47], Al-Kirshi et al., [18], www.feedipedia.org [48]

Table 4. Fatty acids composition of mulberry leaves (% of total FAME)

Specifications*	Mean
Myristic (C14:0)	1.37
Pentadecanoic (C15:0)	2.42
Palmitic (C16:0)	25.22
Heptadecanoic (C17:0)	0.44
Stearic (C18:0)	5.36
Arachidic (C20:0)	1.87
Total SFA	**36.68**
Miristoleic (C14:1)	0.64
Pentadecenoic (C15:1)	7.24
Palmitoleic (C16:1)	2.35
Oleic (C18:1n-9)	3.37
Total MUFA	**13.60**
Linoleic (C18:2n-6)	13.50
α-linolenic (C18:3n-3)	29.07
Octadecatetraenoic (C18:4n-3)	0.20
Eicosatrienoic (C20:3n-6)	0.81
Arachidonic (C20:4n-6)	0.53
Total PUFA	**44.11**
Σ n-6 PUFA	**14.84**
Σ n-3 PUFA	**29.27**
n-6: n-3 ratio	**0.51**

*SFA, saturated FA: (14:0+15:0+16:0+17:0+18:0+20:0); MUFA, monounsaturated FA: 14:1+15:1+16:1+18:1n-9); PUFA, polyunsaturated FA: 18:2n-6+18:3n-3+18:4n-3; 20:3n-6+20:4n-6.
References: Olteanu et al., [43]

According to Srivastava et al. [33], mulberry leaves have much higher levels of beta-carotene (14.688 mg/100 g) compared to other commonly ingested plant leaves (spinach, amaranth, fenugreek). Calcium, potassium, magnesium, phosphorus, iron, boron, manganese, zinc, etc., are important minerals in mulberry leaf composition.

Moreover, mulberry leaves have antioxidants, acid ascorbic [13].

Chlorogenic acid is the most predominant phenolic acid found in mulberry leaves, yet isoquercitrin is the main flavonol glycoside [4].

Additionally, two new flavonoids were found in leaves [49]. Sharma [50] reported also that many phytochemical components are separated from mulberry *Morus rubra* fruit and leaf extract. Several researchers have recently investigated the antioxidant potential of extracts from mulberry plants' leaves, roots, and fruits [51,52].

4. Yeast proprieties

Probiotic yeast's origins can be attributed to Henri Boulard, who isolated the first strain from Indiana fruits in the early 1900s.

According to Ostergaard et al., [53] *S. cerevisiae* is one of the most studied eukaryotic microorganisms, which advances our knowledge of the biology on eukaryotic cells and, eventually, human biology. *S. cerevisiae* has been utilized for several centuries in food and alcohol manufacturing and is currently employed in various pharmaceutical industry activities. *S. cerevisiae* is nonpathogenic and has been considered as safe. *S. cerevisiae* has potential for a variety of biotechnological applications because of its well-established fermentation and process technology for large-scale production and due to its susceptibility to genetic changes [53]. According to Rincon and Benıtez [54], cited by Heitman et al. [55], yeast is a good source of minerals, vitamins (especially B vitamins), lipids, carbohydrates, and amino acids, particularly lysine.

Primary metabolites formed by yeast are acetones, ethanol, proteins, carbons, and amino acids. However, *S. cerevisiae* species also produced several secondary metabolites with exciting properties that are thought to be important for the development of novel chemotherapy medicines. These metabolites include flavonoids, alkaloids, phenolics, and polyketides. Secondary metabolites contribute significantly to organisms functioning even though they are not involved in the primary metabolism of a living cell [56].

5. Yeast metabolism

The term "metabolism" describes how a cell assimilates nutrients through anabolic pathways and dissimilates them through catabolic pathways. Similar to other organisms, yeasts are subject to enzymatic reactions to facilitate these functions [57]. Yeasts are microbial organisms that get their energy (in the form of ATP) from decomposing organic molecules. Understanding the regulatory processes is crucial for biotechnology and the use of the metabolic capacities of specific yeasts as well.

During metabolism, the enzyme-encoding genes and their regulators are expressed in a cellular process through which nutrients are transformed into energy, macromolecular building blocks, and other organic substances [58]. For example, around 20 distinct enzymes are involved in the metabolism of methionine and S-adenosylmethionine.

In yeast, pyruvate is converted by the tricarboxylic acid cycle to CO_2 and water. In the primary and secondary metabolite pathways, acetyl-CoA is a crucial metabolic building block [59].

In the presence of O_2, yeasts usually convert sugars into CO_2, energy, and biomass. In the absence of O_2, yeasts use alcoholic fermentation to turn sugar into ethanol, CO_2, and glycerol [55]. CO_2 and ethanol are the main fermentation products [55,60]. During the fermentation process, yeasts produce from pyruvate various types of compounds, such as isoprenoids, carotenoids, polyketides, polyphenols, lipids, and fatty acids [59].

Regarding nitrogen metabolism, most yeasts can absorb simple nitrogenous sources and use them to biosynthesize proteins and amino acids. A variety of diverse inorganic and organic nitrogen sources, including amino acids (and subsequently peptides and proteins), polyamines, nucleic acids, and vitamins, can be incorporated by yeasts into the cell's structural and functional nitrogenous components.

Glycerol phosphate and fatty acids are the starting points for the biosynthesis of lipids in yeast, which is comparable to processes observed in other species. Yeast lipase breaks down fats to produce glycerol and long-chain fatty acids, which are then catabolized in the glycolytic pathway.

Along with these substances, other secondary metabolites are derived, such as taste compounds, organic acids, glycerol and precursors. These compounds are produced through various metabolic processes, including the glyoxylate cycle, tricarboxylic acid cycle, alcoholic fermentation, and glycolysis [55]. Worthy to mention is the ability of yeast to produce free amino acids (e.g. tryptophan, valine, leucine, phenylalanine) regulated by over 30 enzymes via various pathways, followed by a decarboxylation that can lead to aldehydes generation [60].

By removing feedback inhibition of essential enzymes and by-product creation, *S. cerevisiae* has been metabolically modified to produce aromatic amino acids [59]. When additional species are present, the metabolism of yeast must change and react accordingly [61]. *S. cerevisiae* is highly prevalent in vineyards, but it seems relatively uncommon in natural reservoirs [61,62]. It is interesting to note that insects are natural reservoirs and vectors that aid in the spread of yeast. Regarding interspecies interactions, wine fermentation is arguably the most researched habitat for *S. cerevisiae*.

6. Yeast effects on silkworm *B. mori* L.

Silkworms are insects sensitive to various disease-causing pathogens, environmental (temperature, humidity, hygienic and sanitary conditions) and the nutritional quality of the feed [63,64]. Modern techniques were used to improve mulberry leaves' functional properties and nutritional value for a better economic value for cocoons. Numerous studies have proven that the addition of microbial additives to silkworms can lead to notable results. For example, some studies were conducted to prove the opportunity and advantages of mixing mulberry leaves with certain probiotics to alter silkworm technological parameters [3,64].

A study conducted by Esaivani et al., [3] demonstrated that *S. cerevisiae* improved the cocoon, pupae and shell weight, shell ratio and the filament length, denier, and reeling of the silk. Among the concentrations used, it was demonstrated that the yeast treatment at 5% was significant in increasing the enzyme activity. Thus, the weight of the larvae and the efficiency with which feed is converted into body substances were enhanced by facilitating digestion due to yeast action on amylase and invertase enzyme activity in the digestive juice.

Masthan et al. [6] reported that, in comparison to the control silkworm, the treatment containing 300 ppm of yeast significantly increased cocoon traits, the weight of the pupal, and the length of the silk filament. In addition, there are noteworthy distinctions between the concentrations of 300 ppm and the other two, 100 and 200 ppm. Except for filament length, the differences are not found to be significant at 100 and 200 ppm concentrations.

Yadav and Bagdi [10] in 2016, tested yeast *S. cerevisiae* effects on the rearing performance of the Eri Silkworm. High effects on larval characteristics have been seen at 300 ppm concentration. Second, there are notable distinctions between the concentrations of 300 ppm and the others, which are 100 and 200 ppm. The weight and length of the larvae were also found to differ significantly between concentrations of 100 and 200 ppm. The 300 ppm concentration treatment three times per day increases larval growth.

Taha et al. [7] carried out an experiment wherein two hybrids of silkworms were given probiotics consisting of bacteria (*Bifidobacterium bifidum*) and yeast *S. cerevisiae* mixed with mulberry leaves. Additionally, a strategy to boost *B. mori*'s defence against microbial disease attacks and increase cocoon yield was performed. *Bifidum* and *S. cerevisiae* probiotics greatly enhanced the cocoon characteristics and silk filament, and the impact is more pronounced with *Bifidum.* The influence of *S. cerevisiae* on one of the hybrids, which nearly achieved the same results as *B. bifidum's* on another hybrid, demonstrates how the effects of probiotics differ depending on the hybrid. Using *S. cerevisiae*, one of the hybrids recorded the highest value for pupae weight (1.32±0.02 gm).

Shruti et al., [65] studied silkworm diets based on mulberry leaves treated with four different types of probiotic feed supplements, spirulina, Azolla, yeast, and soy milk, at five different concentrations (1, 2, 3, 4, and 5%), starting in the fourth instar and continuing once a day until the spinning stage. The superior results were recorded for Azolla, followed by soy milk, yeast, and spirulina.

In 2020, Abdelmegeed [64] used mulberry leaves *Morus nigra* enriched with soybeans and yeast *S. cerevisiae* at varying concentrations to boost silkworm cocoon productivity and investigate its impact on fecundity and fertility. In the fifth instar larvae, Abdelmegeed [64] added three different levels of supplements (3, 6 and 9% of soybean or yeast).

Another study done by Soliman, [66], showed that mulberry leaves supplemented with varying concentrations of two probiotics, the blue-green algae *S. platensis* (0.01%, 0.03%, and 0.05%) and the yeast *S. cerevisiae* extract (0.1%, 0.3%, and 0.5%), may have positive and noteworthy effects on the development of *B. mori* larvae as well as improve the characteristics of cocoons. Comparing studies on silkworm larvae using yeast to those using spirulina, the results of the spirulina were inferior to those of yeast, which confirms the results obtained by Shruti [65]. Soliman [66] recommended a 0.5% yeast aqueous solution concentration, enhancing the weight of individual larvae, cocoons, shells, pupae, silk ratio, and total hemolymph protein.

7. Yeast as a functional additive for silkworm

Silkworms are linked to a broad community of symbiotic microorganisms and exhibit a broad range of reliance on gut bacteria for vital functions [67].

Commensal flora is essential to insect survival due to their protection from pathogens microbes through colonial resistance, the production of toxins and the activation of the insects' immune system [68,69].

Infectious gastrointestinal illnesses are known to be influenced by microbiota. In many human medicine studies, intestinal microbiota disequilibrium has been connected to gastrointestinal illnesses such as ulcers, irritable bowel syndrome, antibiotic-associated diarrhoea, inflammatory bowel disease, and colon cancer [70]. According to Staniszewski [71], yeast added to humans' diets can act as a probiotic. *S. cerevisiae spp*. has been used in treating gastrointestinal disorders, including diarrhoea, Crohn's and inflammatory intestinal disease, irritable intestinal syndrome, acute gastroenteritis, and Vibrio cholerae. Yeast has a valuable influence on decreasing the severity of toxocariasis and is also utilized to lessen the adverse effects of Helicobacter pylori treatments [70,71].

In the literature, multiple strategies are underway to enhance silkworm health.

L. plantarum, L. rhamnosus, L. paracasei, and *L. acidophilus* are abundant in the silkworm midgut, followed by Enterococcus and Bacillus. Microorganisms that occur in the intestinal tract release the enzymes responsible for the degradation of cellulose, starch, protein, pectin, xylan, lipids, and fatty acids. The growth, development, and disease resistance of silkworms are known to be influenced by the digestive enzymes that supply vital nutrients or support a crucial biochemical process associated with feed intake [69].

The adaptation of *B. mori* for nutrients from feed supplement and their impact on the type and concentration of the enzymes and morphological/ technological parameters were described by Esaivani et al. [3].

Many diseases can affect silkworms due to various factors, including nutrition, hygienic conditions, and pathogens. Diseases affecting silkworms lead to a 10–20% decrease in sericulture production. Adopting some biotechnology technologies can help alleviate challenges caused by various diseases.

The addition of microbial additives to silkworm diets contributes to the remodeling of gut microbiota [72] and improves health status [69]. However, alterations in the gut microbiota of the silkworms resulted in altered host immunological resistance and nutrition metabolism when they switched from eating mulberry leaves to an artificial diet.

Given the vital role of gut microflora in providing numerous benefits to the insect host, gut-associated bacteria *open doors* for industrial applications in various industries, including biotechnology, agriculture, and disease therapy [68].

There are still many unanswered concerns regarding the symbiotic-host relationship, and modelling/prediction tools are required to explore how nutrition, environmental factors, and microbiota affect these interactions.

8. Conclusions

Even though mulberry leaves are nutritionally rich and are considered a complete feed for silkworms, the fortification of their active principles by using yeasts can be a valuable tool leading to improving the productivity of the sericulture sector. As mulberry leaves, yeast (*S. cerevisae*) is a good source of minerals, vitamins (especially B vitamins), lipids, carbohydrates, and amino acids, particularly lysine. Yeast can act as a probiotic, releasing digestive enzymes and modelling gut microbiota. Alterations in the gut microbiota of the silkworms lead to an improvement of the host immunological resistance and nutrition metabolism that implies avoiding diseases. Therefore, technological/morphological silkworm parameters can be seriously ameliorated.

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References

1. Muzamil, A., Tahir, H. M., Ali, A., Bhatti, M. F., Munir, F., Ijaz, F., Adnan, M., Khan, H. A., Qayyum, K. A., Effect of amino acid fortified mulberry leaves on economic and biological traits of *Bombyx mori* L., Heliyon, 2023, 9(10), E21053, doi.org/10.1016/j.heliyon.2023.e21053.

2. Jan, B., Parveen, R., Zahiruddin, S., Khan, M. U., Mohapatra, S., Ahmad, S., Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review. Saudi Journal of Biological Science, 2021, 28 (7), 3909-3921. doi.org/10.1016/j.sjbs.2021.03.056.

3. Esaivani, C., Vasanthi, K., Bharathi, R., Chairman, K., Impact of probiotic saccharomyces cerevisiae on the enzymatic profile and the economic parameters of silkworm *Bombyx mori* L, Advances in Biology &BioMedicine, 2014, 1 (1).

4. Chuah, H. Q., Tang, P. L., Ang, N. J., Tan, H. Y. Submerged fermentation improves bioactivity of mulberry fruits and leaves, Chinese Herbal Medicines, 2021, 13, 4, 565-572.

5. Alam, K., Raviraj, V. S., Chowdhury, T., Bhuimali, A., Ghosh, P., Saha, S., Application of biotechnology in sericulture: Progress, scope and prospect, Nucleus, 2021, 65(1), 129–150, doi.org/10.1007/s13237-021-00355-2.

6. Masthan, K., Rajkumar,T., and Narasimhamurthy, C. V., Beneficial effects of blue green algae Spirulina and yeast Saccharomyces cerevisiae on cocoon quantitative parameters of silkworm,*Bombyx mori* L., Asian Journal of Microbiology,Biotechnology and Environmental Sciences, 2011, 13(1), 205 -208.

7. Taha, R. H., Soliman, S. A. and Kamel, H. M. Micro-Organisms Supplementation to Mulberry Silkworm, *Bombyx mori* L, Egyptian Academic Journal of Biological Sciences, 2017, 10(2), 57-64, ISSN 1687-8809.

8. Anisha, S. P., Yasmin, N., Devi, E. S. V., Supplementation of mulberry leaves with probiotic bifilac on economic traits of silkworm (*Bombyx mori*), International Journal of Food Science and Nutrition, 2022, 11 (12), 86-90, e-ISSN 2320–7876 www.ijfans.org.

9. Amala, R. G.; Padmalatha, C., and Ranjith Singh, A. J. A., Probiotic supplementations to improve commercial characteristics, disease resistance and protein in the silkworm *Bombyx mori* L. World Journal of Biological Research, 2011, 4(2), 12-25.

10. Yadav, U., and Bagdi, A., Supplementary effect of yeast (*Saccharomyces Cerevisiae*) on rearing performance of Eri Silkworm (*Philosamia Ricini*) in respect of some larval parameter, Environmental Conservation Journal, 2016, 17(1&2) 95-98.

11. Tang, R., Zhang, F., Zhang, Z.N., Electrophysiological responses and reproductive behavior of fall webworm moths (hyphantria cunea drury) are influenced by volatile compounds from its mulberry host (*Morus alba* L.), Insects, 2016, 7, 19, doi:10.3390/insects7020019.

12. Tanaka, K., Uda, Y., Ono, Y., Nakagawa T., Suwa M., Yamaoka R., and Touhara K. Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile, Current Biology, 2009, 19, 881–890. doi.10.1016/j.cub.2009.04.035.

13. Hăbeanu, M., Gheorghe, A., Mihalcea T., Șapcaliu A., Savu, V., Măicănescu, M. Nutritive value, use and importance of mulberry leaf for the silkworm *Bombyx mori* L., Acta Agricola Romanica, 2023, 5, 5.3. 56-64.

14. Hosseini, A. S., Akramian, M., Khadivi, A., Arjmand, H. S., Phenotypic and chemical variation of black mulberry (*Morus nigra*) genotypes, Industrial Crop and Product, 2018, 117, 260–271.

15. Krishna, H., Singh, D., Singh, R. S., Kumar, L.; Sharma, B. D., Saroj, P. L. Morphological and antioxidant characteristics of mulberry (*Morus spp*.) genotypes, Journal of the Saudi Society of Agricultural Sciences, 2020, 19(2), 136-145. doi.org/10.1016/j.jssas.2018.08.002.

16. Alipanah, M., Abedian, Z., Nasiri, A., and Sarjamei, F., Nutritional Effects of Three Mulberry Varieties on Silkworms in Torbat Heydarieh, Psyche: A Journal of Entomology, 2020, 1-4. doi.org/10.1155/2020/6483427

17. Chundang, P., Thongprajukaew, K., Kovitvadhi, U., Chotimanothum, B., Kovitvadhi, A., Pakkong, P., Improving the nutritive value of mulberry leaves, Morus spp. (Rosales: Moraceae) for silkworm larvae, Bombyx mori (Lepidoptera: Bombycidae) using gamma irradiation, Journal of Radiation Research and Applied Science, 2020, 13, 1, 629–641, doi.org/10.1080/16878507.2020.1820268.

18. Al-Kirshi, R. A., Alimon, A., Zulkifli, I., Atefeh, S., Zahari, M. W., & Ivan, M. Nutrient digestibility of mulberry leaves (*Morus alba*). Italian Journal of Animal Science, 2013, 12, 219–221, doi.org/10.4081/ijas.2013.e36.

19. Raghuvanshi, T., Bali, R. K., and Bukhari, R., Effect of Different Feeding Frequencies on the Commercial Characters of Silkworm (*Bombyx mori L.*). International Journal of Current Microbiology and Applied Sciences, 2019, 8(6), 3193-3203.doi.org/10.20546/ijcmas.2019.806.381.

20. Sanchez-Salcedo, E. M., Amoros, A., Hernandez, F., Martinez, J. J., Physicochemical properties of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves, a new food supplement. Journal of Food Nutrition and Research 2017, 5, 253–261.

21. Thaipitakwong, T., Numhom, S., & Aramwit, P., Mulberry leaves and their potential effects against cardiometabolic risks: a review of chemical compositions, biological properties and clinical

efficacy, Pharmaceutical Biology, 2018, 56(1), 109-118, doi: 10.1080/13880209.2018.1424210.
22. Iqbal, S., Younas, U., Sirajuddin, Chan, K. W., Sarfraz, R.A., Uddin, K., Proximate composition and antioxidant potential of leaves from three varieties of mulberry (*Morus sp*.): A comparative study, International Journal of Molecular Sciences, 2012, 13, 6651–6664.
23. Adeduntan, S.A., Oyerinde, A. S., Evaluation of nutritional and antinutritional characteristics of obeche (Triplochition scleroxylon scleroxylon) and several mulberry (*Morus alba*) leaves, African Journal of Biochemistry Research, 2010, 4, 175–178.
24. Muzamil, A., Tahir, H. M., Ali, A., Bhatti, M. F., Munir, F., Ijaz, F., Adnan, M., Khan, H. A., Qayyum, K. A, Effect of amino acid fortified mulberry leaves on economic and biological traits of *Bombyx mori* L., Heliyon, 2023, 9 (10), e21053.
25. Alidee, T., Jales, M., Hjaij, N. D., and Almouna, A., Estimation of Some Phytochemical Compounds and Antioxidant Properties of Leaves from Different Mulberry Varieties Grown in Syria, Hindawi Evidence-Based Complementary and Alternative Medicine, 2023, 2023, 1-6, doi.org/10.1155/2023/9929276.
26. Kumar, D., Dev, P., and Kumar, R. V., Biomedical Applications of Silkworm Pupae Proteins. Biochemistry and Molecular Biology, Biomedical Applications of Natural Proteins., 2015, pp.41–49, *(Chapter 3)*. doi:10.1007/978-81-322-2491-4_3.
27. Chang, B. Y., Koo, B-S., and Kim, S. Y., Pharmacological activities for *Morus alba* L., focusing on the immunostimulatory property from the fruit aqueous extract, 2021, 10, 1966. doi.org/10.3390/foods10081966.
28. Imran, M., Khan, H., Shah, M., Khan, R., Khan, F., Chemical composition and antioxidant activity of certain Morus species, 2010 11(12):973-980.
29. Dolis, M. G., Simeanu, C.., Usturoi, A., Simeanu, D., Research regarding chemical composition and the digestibility of the mulberry leaves from Eforie variety, Revista de Chimie (Bucharest), 2017, 68, 1. 151-156.
30. Doliș, M. G., Donose, R., Simeanu, C., Usturoi, A., Rațu, R., Research regarding chemical composition of the mulberry leaves from Kokuso 21 variety, Analele Universității din Oradea, Fascicula: Ecotoxicologie, Zootehnie și Tehnologii de Industrie Alimentară 2016, XV/A 207-212.
31. Doliș, M. G., Rațu, R. N., Study regarding the use of mulberry leaves (Kokuso 21variety) *by Bombyx mori* (Triumf hybrid), Lucrări Ştiinţifice – seria Agronomie, 2019, 62(1), 65-72.
32. Vu, C. C. , Verstegen, M. W. A., Hendriks, W. H. and Pham, K. C., The nutritive value of mulberry leaves (*Morus alba*) and partial replacement of cotton seed in rations on the performance of growing vietnamese cattle, Asian-Australian Journal of Animal Science, 2011, 24, 9, 1233 – 1242.
33. Srivastava, S., Kapoor, R., Thathola, A., and Srivastava R. P., Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). International Journal of Food Science and Nutrition, 2006, 57(5/6), 305313. doi: 10.1080/09637480600801837.
34. Kandylis, K., Hadjigeorgiou, I., Harizanis, P., The nutritive value of mulberry leaves (Morus alba) as a feed supplement for sheep, Tropical Animal Health and Production, 2009, 41,17–24, doi 10.1007/s11250-008-9149-y.
35. Yu, Y., Li, H., Zhang, B., Wang, J., Shi, X., Huang, J., Yang, J., Zhang, Y., & Deng , Z., Nutritional and functional components of mulberry leaves from different varieties: Evaluation of their potential as food materials, International Journal of Food Properties, 2018, 1 (1), 1495-1507, doi:10.1080/10942912.2018.1489833.
36. Astuti, D. A., Becker, K., Richter, N., Energy and protein balance of Nile tilapia fed with moringa and mulberry leaves, Jurnal Pengolahan Hasil Perikanan Indonesia, 2012, 5(1).
37. Kang, K., Wang, R., Tang, S., Wang, M., Tan, Z., Bernard, L. A., Chemical composition and in vitro ruminal fermentation of pigeonpea and mulberry leaves, Agroforest Syst, 2019, doi.org/10.1007/s10457-019-00410-7.
38. Sahoo, A., Singh, B., Sharma, O. P., Evaluation of feeding value of Eupatorium adenophorum in combination with mulberry leave, Livestock Science, 2011, 136, 2–3, 175-183, https://doi.org/10.1016/j.livsci.2010.08.019.
39. Ustundag, A. O., Ozdoga, M., Usage possibilities of mulberry leaves in poultry nutrition, Scientific Papers. Series D. Animal Science, 2015, LVIII, 170-178.
40. Machii, H., and Katagiri, K., Varietal differences in nutritive values of mulberry leaves for rearing silkworms, Japan Agricultural Research Quarterly, 1991, 25, 202-208.
41. Machii, H., Koyama, A., Yamanouchi, H., Mulberry breeding, cultivation and utilization in Japan, FAO Electronic Conference on Mulberry for animal production (Morus 1-l), 2002. [accessed on January, 2024].
42. Yao, J., Yan, B., Wang, X.Q., and Liu, J. X., Nutritional evaluation of mulberry leaves as feed for ruminants. Livestock Researc Rural Development 2000, 12 (2). [Accessed on January, 2024].
43. Olteanu M., Criste, R. D., Cornescu, G. M., Ropota M., Panaite, T., Varzaru, I., Effect of dietary mulberry (Morus alba) leaves on performance parameters and

quality of breast meat of broilers, Indian Journal of Animal Science, 2015, 85 (3), 291–295.
44. Wang, C., Yang, F., Wang, Q., Zhou, X., Xie, M., Kang, P., Wang, Y., Peng, X., Nutritive value of mulberry leaf meal and its effect on the performance of 35-70-day-old geese, Journal of Poultry Sciences, 2017, 54, 41-46.
45. Al-kirshi, R. A., Alimon, A. R., Zulkifli, I., Zahari, M. W., and Sazil, A. Q., The chemical composition and nutritive value of mulberry leaf as a protein source in poultry diets. The 1st International Seminar on Animal Industry, Feed and Nutrition 2009, 98-102. [accesed on January 25, 2024].
46. Adeduntan, S. A., and Oyerinde, A. S., Evaluation of chemical and antinutritional characteristics of obeche (Triplochition scleroxylon) and some mulberry (Morus alba) leaves, International Journal of Biological and Chemical Science, 2009, 3(4), 681-687.
47. Bamikole, M.A., Iknatua, M.I., Iknatua, U.J., Ezenwa, I.V., Nutritive value of mulberry (Morus spp) leaves in the growing rabbits in Nigeria, Pakistan Journal of Nutrition, 2005, 4(4), 231-236.
48. www.feedipedia.org [Accessed on January, 2024].
49. Gao, L., Li, Y.-D., Zhu, B.-K., Li, Z.-Y., Huang, L.-B., Li, X.-Y., Wang, F., Ren, F.,-C., Liao, T.-G. Two new prenylflavonoids from *Morus alba*. Journal of Asian Natural Products Research, 2017, 1–5. doi:10.1080/10286020.2017.1343303.
50. Sharma, S.B., Tanwar, R.S., Rini, A.C., Singh, U.R., Gupta, S., and Shukla, S.K., Protective effect of *Morus rubra* L. leaf extract on diet-induced atherosclerosis in diabetic rats, Indian Journal of Biochemistry and Biophysic, 2010, 47: 26-31.
51. Andallu, B., Varadacharyulu, N. Ch., Control of hyperglycemia and retardation of cataract by mulberry (*Morus indica* L.) leaves in streptozotocin diabetic rats, Indian Journal of Experimental Biology., 2002, 40(7), 791-5. PMID: 12597548.
52. Arfan, M., Khan, R., Rybarczyk, A., and Amarowicz, R., Antioxidant activity of mulberry fruit extracts, International Journal of Molecular Sciences, 2012, 13, 2472-2480. doi:10.3390/ijms13022472.
53. Ostergaard, S., Olsson, L., and Nielsen, J., Metabolic Engineering of *Saccharomyces cerevisiae*, Microbiology and Molecular Biology Reviews, 2000, 64(1), 34–50.
54. Rincon, A. M., and Benıtez, T., Improved organoleptic and nutritive properties of bakery products supplemented with amino acid overproducing Saccharomyces cerevisiae yeasts, Journal of Agriculture and Food Chemistry, 2001, 49,1861–1866.
55. Heitmann, M., Zannini, E., & Arendt, E., Impact of Saccharomyces cerevisiae metabolites produced during fermentation on bread quality parameters: A review, Critical Review in Food Science and Nutrition, 2018, 58, 7, 1152–1164, doi.org/10.1080/10408398.2016.1244153.
56. Jahangeer, M., Riasat, A., Mahmood, Z., Numan, M., Munir, N., Ashiq, M., Asad, M., Ali, U., and Salman, M., Secondary Metabolites from Saccharomyces cerevisiae Species with Anticancer Potential, Saccharomyces, Intech Open, 2021, doi: 10.5772/intechopen.95067.
57. https://onlinelibrary.wiley.com/doi/10.1002/9783527659180.ch3. Book Editor(s): Horst Feldmann, Yeast Metabolism, Yeast, 2012. 25–58. Yeast: Molecular and Cell Biology, Second Edition. Wiley-VCH Verlag GmbH & Co. KGaA. Published 2012 by Wiley-VCH Verlag GmbH & Co. KGaA. Chapter 3. doi:10.1002/9783527659180.ch3. [Accessed on March 2024].
58. Bradley, P. H., Brauer, Matthew, J. Rabinowitz, J. D., Troyanskaya, O. G., Coordinated concentration changes of transcripts and metabolites in *Saccharomyces cerevisiae*, PLoS Computational Biology, 2009, 5, 1, e1000270.
59. Chrzanowsk, G., *Saccharomyces Cerevisiae*—an interesting producer of bioactive plant polyphenolic metabolites, International Journal of Molecular Science, 2020, 21, 7343; doi:10.3390/ijms21197343.
60. Pronk, K. T., Steensmays, H. Y., Van Dijkent, J. P., Pyruvate Metabolism in *Saccharomyces cerevisiae*, yeast, 1996, 12, 1607-1633.
61. Jouhten, P., Ponomarova, O., Gonzalez, R. and Patil, K. R., *Saccharomyces cerevisiae* metabolism in ecological context, FEMS Yeast Research, 2016, 16, fow080, doi: 10.1093/femsyr/fow080.
62. Goddard, M. R., Greig, D., *Saccharomyces cerevisiae*: a nomadic yeast with no niche? FEMS Yeast Research, 2015,15, fov009.
63. Moustafa, M. N., and Soliman, S. A., Nutritional efficiency and economic traits of silkworm Bombyx mori, l. reared on mulberry leaves fortified with synbiotics, Journal of Plant Protection and Pathology, 2019, 10 (12), 671-675, doi: 10.21608/jppp.2019.79460.
64. Abdelmegeed, S. M., Biological and Physiological Effects on Mulberry Silkworm Fed on Mulberry Leaves Treated with Yeast and Soybean, Journal of Plant Protection and Pathology, 2020, 11(7), 349-351, doi: 10.21608/jppp.2020.108895.
65. Shruti, Ashoka, J., Hadimani, D. K., Sreeniva,s A. G., and Beladhadi, R. V., Effect of probiotic feed supplements to mulberry silkworm, Bombyx mori L. for larval growth and development parameters, International Journal of Chemical Studie, 2019; 7(3), 3914-3919.
66. Soliman, A. M., The Impact of Fortification of Mulberry Leaves with the Yeast Saccharomyces cerevisiae and the Blue Green Algae Spirulina platensis on some Quantitative Parameters of Silkworm Bombyx mori (L.), Journal of Plant Protection and Pathology, 2021, 12 (1), 55 - 59, doi: 10.21608/jppp.2021.58388.1011.

67. Savio, C., Mugo-Kamiri, L., and Upfold J. K., The Role of Probiotics and Prebiotics in Maintenance of Health in Mass-Reared Insects, Insects, 2022, 13, 376. doi.org/10.3390/ insects13040376.
68. Barretto, D. A., Gadwala, M., Vootla, S. K., The silkworm gut microbiota: A potential source for biotechnological applications, Methods in Silkworm Microbiology, 2021, 1-26, doi:10.1016/bs.mim.2021.04.001.
69. Arasakumar, V., Vasanth, E., Vijay, S., and Swathiga, G., Role of microorganisms in the gut of silkworms, The Pharma Innovation Journal, 2023, 12(4), 2691-2696.
70. Czerucka, D., Piche, T., & Rampal, P., Review article: yeast as probiotics – Saccharomyces boulardii, 73. Alimentary Pharmacology & Therapeutics, 2007, 26, 767–778. doi:10.1111/j.1365-2036.2007.03442.x.
71. Staniszewski, A., Kordowska-Wiater, M., Probiotic and potentially probiotic yeasts— characteristics and food application, Foods, 2021, 10, 1306. doi.org/ 10.3390/foods10061306
72. Yuan, S., Sun, Y., Chang, W., Zhang, J., Sang, J., Zhao, J., Song, M., Qiao, Y., Zhang, C., Zhu, M., Tang, Y., & Lou, H., The silkworm (Bombyx mori) gut microbiota is involved in metabolic detoxification by glucosylation of plant toxins, Communications Biology, 2023, 6, 790 doi.org/10.1038/s42003-023-05150-0.