Essential Micronutrient Biofortification of Sprouts Grown on Mineral Fortified Fiber Mats

Jacquelyn Nyenhuis, Jaroslaw W. Drelich

Abstract**—**Diets high in processed foods have been found to lack essential micro-nutrients for optimum human development and overall health. Some micro-nutrients such as copper (Cu) have been found to enhance the inflammatory response through its oxidative functions, thereby having a role in cardiovascular disease, metabolic syndrome, diabetes and related complications. This research study was designed to determine if food crops could be bio-fortified with micro-nutrients by growing sprouts on mineral fortified fiber mats. In the feasibility study described in this contribution, recycled cellulose fibers and clay, saturated with either micro-nutrient copper ions or copper nanoparticles, were converted to a novel mineral-cellulose fiber carrier of essential micro-nutrient and of antimicrobial properties. Seeds of *Medicago sativa* (alfalfa), purchased from a commercial, organic supplier were germinated on engineered cellulose fiber mats. After the appearance of the first leaves, the sprouts were dehydrated and analyzed for Cu content. Nutrient analysis showed ~2 increase in Cu of the sprouts grown on the fiber mats with copper particles, and ~4 increase on mats with ionic copper as compared to the control samples. This study illustrates the potential for the use of engineered mats as a viable way to increase the micro-nutrient composition of locally-grown food crops and the need for additional research to determine the uptake, nutritional implications and risks of micro-nutrient bio-fortification.

Keywords**—**Bio-fortification, copper nutrient uptake, sprout, mineral-fortified mat, micro-nutrient uptake.

I. INTRODUCTION

HEALTH benefits of essential micro-nutrients such as copper have been substantiated by documentation of improved heart health and the ability to help maintain overall health status [1], [2]. Highly processed diets lack essential micronutrients which are important for growth, development and overall health [3], and at the same time, ultra-processed foods have steadily replaced minimally processed foods in the US and throughout the world [4]. The shift of this food culture paradigm gives increased importance on developing ways to preserve, increase and enhance intake of essential micronutrients. Sprouts germinated from seeds not only have a high nutrient content in terms of essential micronutrients but research has also established a potential protective effect against cancer of consumable sprouts [5].

Sprouts were grown on specially engineered mineral fortified fiber mats in this research study, designed to determine if food crops could be bio-fortified with micronutrients. Primary objective is to develop a rapid and simple method of increasing the micronutrient composition of sprouts that could be used at home or developed for the commercialization of biofortification of food crops. Sprouts themselves could be of value as a source of fresh produce economically grown anywhere, any season and with a very small footprint. The sprouting of seeds does not require sun or soil so they can be grown indoors. The time from germination to a consumable sprout is a relatively short 4 or 5 day period. With the increased emphasis on nutritive value of foods and especially that of so called natural foods, this study was designed to analyze if copper was picked up by the plants from the structure of cellulose fiber mats embedded with either copper nanoparticles or copper ions.

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II. EXPERIMENTAL

A commercial vermiculite product from Virginia Vermiculite LLC, Milled No.7 was used in this study (Fig. 1). This vermiculite is an exfoliated powder product produced by thermal exfoliation from vermiculite concentrate. It is also a sheet platelet with a bulk density of 96-160 kg/m^{3}and a cation exchange capacity of 50-150 mmol/100g.

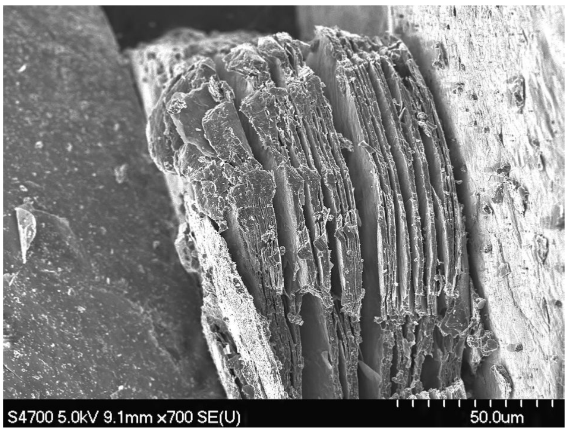

Fig. 1 Scanning electron microscopy (SEM) micrograph of exfoliated vermiculite used in this study

The vermiculite was saturated with copper (Cu) ions to the amount of ~4.8 wt% by cation exchange process in 1 M $CuSO_4$ solutions at 90^{o}C [6]. Formulated copper ion – saturated vermiculite, after several washings with deionized water and drying, was split for two batches: one used as a filler for manufacturing mats and one underwent additional reduction process. Reduction process took place in a hydrogen atmosphere at 600°C and was carried out to convert ionic copper to metallic nanoparticles.

Samples of both Cu ion-saturated vermiculite and vermiculite decorated with Cu nanoparticles were soaked in water in ratio of 10 gram per 1L for 24 hours to determine the amount of released copper. The content of Cu ions in water was determined through Inductively Coupled Plasma Spectroscopy (ICP) analysis.

Mineral-fortified cellulose fiber mats were prepared by adding copper-based vermiculites to 1 wt% water-based paper pulp in a laboratory mixer and homogenizing the mixture for 5 minutes. After mixing, water was removed from the pulp by vacuum filtration and drying at 70-80°C for several hours. The content of copper ion-based vermiculite or vermiculite decorated with copper nanoparticles was kept constant in the mat at 11 wt%.

Medicago sativa, also known as lucerene, is called alfalfa in the United States. For this study, alfalfa seeds commonly distributed commercially for sprouting were prepared for germination by soaking 25 grams of seeds in ~400ml of distilled water for 12 hours.

Prior to germination the engineered cellulose fiber mats were soaked in distilled water for 10 minutes and placed on a glass plate to catch excess water. The seeds were drained and spread over each engineered cellulose fiber mat on a plate and then allowed to sprout at ambient room temperature (19 to 28°C) [7] for 120 hours under the usual light and dark conditions during day and night. The moist atmosphere was achieved by placing the engineered cellulose fiber mats on a glass plate which collected the excess water from soaking the mats. The excess water was allowed to pool under the mats and became a reservoir of water that the mats could take up as moisture evaporated from the surface. The seeds, dispersed across the mats, were covered loosely with a plastic film that was lifted daily to prevent unwanted moisture build-up or to be sprayed with additional water to keep the seeds at an appropriate moisture level without overwatering them. After the appearance of the first full set of leaves, the sprouts were washed with distilled water [8]. The samples were dried in an oven at 65°C for 10 hours, ashed and then dissolved in hydrochloric acid. Solutions after dilution with deionized water were analyzed for copper content using ICP analysis.

III. Results

Aluminosilicate mineral used in this study, vermiculite, has a layered structure (Fig. 1) and high cation exchange capacity similar to other clay minerals. It is an ideal carrier material for loading Cu ions [9], [6], and converting them to metallic nanoparticles [6]. The cation exchange capacity of typical vermiculite is between 100 mmol/100g and 150 mmol/100g [9]; that is, vermiculite can absorb 6.4-9.5 wt% of Cu ions. Ion exchange process is however, very slow and this process was stopped after loading Cu ions to 4.8 wt%. This product was used to prepare the first batch of mineral-fortified cellulose mats that we will call V-Cu^{2+} in the next part of this contribution.

Copper ions were also reduced in hydrogen environment to copper nanoparticles in one portion of vermiculite product from the ion exchange process. Nanoparticles with diameters ranging from a few to over 500 nm are typically produced in the structure of vermiculite as described in details in [6]. As shown in Fig. 2, typical size of copper nanoparticles residing on surfaces of vermiculite processed in this study was in the range of 50 to 300 nm. Detailed particles size distribution was not analyzed. This product was used to prepare the second batch of mineral-fortified cellulose mats that we will call V-Cu^0 in the next part of this contribution.

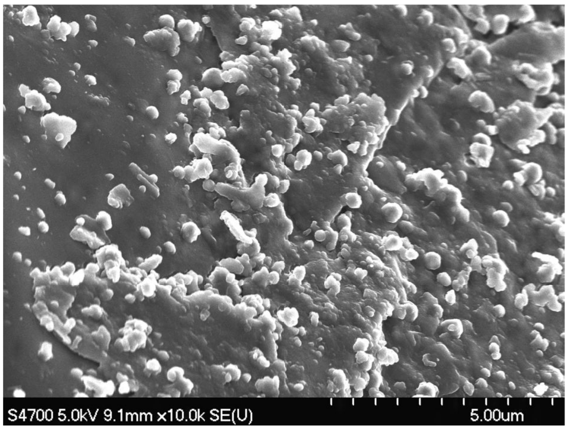

Fig. 2 SEM micrograph showing a vermiculate particle surface decorated with copper nanoparticles

It was determined that both vermiculite samples, with Cu ions and Cu nanoparticles, leached copper ions to water during their soaking. The content of Cu ions in water used to soak V-Cu^{2+} and V-Cu^0 was 3+/-2 ppm and 0.2+/-0.1 ppm, respectively.

Since vermiculite is a clay it could be easily incorporated to paper pulp to prepare mats. The experimental mats incorporated ~11 wt% clay fillers saturated with either Cu nanoparticles or ionic Cu and then allowed to dry. Fig. 3 shows the image of zoomed section of one of the mats used in this study. Dark specs represent vermiculite particles embedded in the structure of cellulose fibers.

Fig. 3 Optical image of vermiculite-fortified cellulose mat

Cellulose fiber mats were engineered using recycled cellulose fibers and clay capable releasing micro-nutrient ions of copper. Fig. 4 shows example of alfalfa sprouts germinated on one of the mats.

Fig. 4 Sprouts that grew on mineral-fortified cellulose mat

Increase in copper in the sprouts grown on the fiber mats with copper nanoparticles was ~2x, and nearly 4 times increase on mats with ionic copper as compared to the control samples (Table I).

TABLE I
SAMPLES TESTED AND AMOUNT OF COPPER IN ALFALFA SPROUTS (ND – NOT DETERMINED)

Sample	Composition of mats			Copper content in plants [mg/kg dry]
	Mineral content [wt%]	Cu content [wt%]	Cu form	
Cellulose mat	0	ND	ND	5 ± 1
V-Cu^0	11	0.5	Nano	11 ± 4
V-Cu^{2+}	11	0.5	Ionic	20 ± 7

IV. DISCUSSION

Aluminosilicate mineral used in this study, vermiculite, is commonly used in agriculture and horticulture as potting material, seed and fertilizer carier, and soil conditioner. Additionally, as we have demonstrated in this contribution, it could be used as a filler to novel cellulose fiber-based mats that serve as a potting bed for germination and growth of micro-greens capable of holding water and suppling extra micronutrients. With the USDA mandate that Americans should consume more fruits and vegetables, producers and manufacturers are interested in marketing a wider variety of produce including sprouts which have been known to have increased nutrient value [10]-[12]. Copper, an essential micronutrient was used as an example in this feasibility study and saturation of mats with vermiculite carrying other micronutrients could easily be expended.

Micronutrients are substances that are needed in comparatively miniscule amounts for optimal health. There is a whole range of micronutrients that contribute to normal body function, some of the important contributions being that they have antioxidative properties and may be involved in neuro-hormonal signaling [13]. Enhancement of the inflammatory response through its oxidative functions, copper thereby has a role in cardiovascular health, metabolic syndrome, diabetes and related complications [14]. Importantly, existing data shows that while copper deficiency was rare in the past, micronutrient malnutrition affects a staggering half of the world's population [15] and trends such as a Westernized diet and popularity of gastric bypass surgery are correlated to impaired copper status [16], [17]. Signs of copper deficiency can be seen for years after bypass surgery and includes moderate to severe neurological complications [18]. Currently, supplementation of micronutrients is a wide-spread practice for patients found to be deficient [19]. An alternative is to increase the micronutrient content of foods.

Bio-fortification has been proposed as a way to increase micronutrients such as copper that might be missing in the human diet [20]. Sprouts are known to have increased levels of phytochemicals [21] and could be a natural carrier for increased micronutrients through bio-fortification. Therefore, the objective was fulfilled for this study and the sprouts grown on engineered cellulose fiber mats gained 2 to 4 times more copper than sprouts grown on cellulose fiber mats without filler with copper.

This study was limited to copper-containing vermiculite samples with constant copper content. Although the spectrum of tests was limited, this study clearly shows that the supply of micronutrients such as copper can be controlled through engineering of mats. For example, the release of Cu from vermiculite saturated with Cu^{2+} ions is about one order of magnitude faster than from vermiculite decorated with metallic nanoparticles. As the result, the sprouts grown on the V-Cu^{2+} mats carry nearly two times more copper than sprouts germinated on the V-Cu^0 mats. This probably could be further manipulated by the content of vermiculate in the structure of cellulose fiber mats, although the correlation between Cu content in mats and sprouts would need to be confirmed through detailed experimentation.

An important additional benefit of the engineered cellulose fiber mats is the well-known antibacterial properties of Cu. Copper as an antimicrobial agent has been used in many applications even before its antimicrobial properties were fully understood and even before the recognition of the existence of pathogenic microorganisms. Since then, the antimicrobial properties of Cu have been instrumental in a wide range of applications, from hygienic medical devices to antiseptics to antimicrobial medicines. Studies have demonstrated that copper and copper alloys can reduce the toxicity of pathogenic microorganisms that cause 4 to 6 million deaths per year [22]. Examples of enteric bacteria (geo-treatable) that copper substances can reduce the transmission of include *Escherichia coli*, *Listeria monocytogenes Salmonella enterica,* and others, which are highly toxic [23]. Sprouts infected with any of these toxic microorganisms can potentially become a public health disaster if not detected. Toxic microorganisms can be transmitted to sprouts when the seed itself is contaminated, the water is contaminated or the growing medium is contaminated. Fiber mats with imbedded copper prevents the growing surface from being contaminated and current research indicates that copper will also reduce the transmission of toxic bacteria that might be carried by the seed or come in contact with the seed of the sprout grown on the fiber mats.

The antibacterial properties of vermiculite saturated with Cu ions and Cu nanoparticles have been verified with halo method tests against *Escherichia Coli* in previous studies [9], [6]. *E. coli* is a known pathogenic risk in sprout production. Copper exhibits excellent antibacterial properties when tested on *S. aureus*, a pathogenic gram-positive bacterium.

V. Conclusion

A novel cellulose-clay hybrid material which is embedded with either ionic copper or nanoparticles of elemental copper can be used as a growing medium for sprouts. Nutrient analysis of the sprouts showed a significant increase in copper content of the sprouts compared to sprouts grown on control cellulose fiber mats. The uptake, bio-fortification and risks of nano-materials and ions are not well understood but the use of engineered mats might be a sustainable and cost-effective way to alleviate mineral deficiency in diverse populations.

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