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Research Article

Antimicrobial Effect of Basil Leaf Extract (*Ocimum sanctum* L.) to Preserve the Quality of Wet Noodles

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Abstract

Background and Objective: Basil leaves (*Ocimum sanctum* L.) contain fungicidal and antibacterial compounds such as linalool (56.7-60%) which can be applied as a natural preservative for food including wet noodles. The purpose of this study was to determine the appropriate concentration of basil leaf extract that could inhibit the growth of *Escherichia coli* and *Aspergillus niger* to increase the shelf life of wet noodles. **Materials and Methods:** This study ran an experimental method pursued by statistical analysis using 2 experiments with duplication. Each experiment included the determination of the antimicrobial activity of basil extract (0, 25, 50, 75 and 100% of concentration) against *E. coli* and *A. niger* by using the agar diffusion method. The next experiment tested antimicrobial activity using the best concentration of basil extract in wet noodles. **Results:** The results of this study exhibited that a 100% concentration of basil leaf extract could inhibit *E. coli* and *A. niger* with inhibition zones of 30.98 and 5.96 mm, respectively. Antimicrobial activities of 100% concentration of basil extract in wet noodles showed the total number of moulds and coliform values were 3.8×10^9 CFU g⁻¹ and <10 APM/g, respectively, until 3 days after treatment. Thus, the wet noodles treated with basil leaf extract exhibited good flavour and colour. **Conclusion:** This study concluded that the treatment of basil leaf extract inhibited *E. coli* and *A. niger* activities and preserve the quality of wet noodles.

Key words: Antimicrobial, antifungal, basil leaf extract, natural preservative, wet noodles, flavonoids, steroids

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food safety is an important health issue. Unsafe foods can cause or contribute to many illnesses, from diarrhoea to some types of cancer. Food safety is a crucial food issue to prevent biological, chemical and other contaminations from food¹. Therefore, this is an important thing to implement to improve food quality. Wet noodle has a short shelf life because it has relatively high water content highly susceptible to being contaminated by either microorganism such as *Pseudomonas*, *Aeromonas*, *Bacillus*, *Streptococcus* and *Staphylococcus* sp. or fungus such as *Candida*, *Aspergillus*, *Rhodotorula*, *Penicillium* and *Mucor*².

Indonesia is one of the largest producers of spices, a natural preservative, in the world. As a spice producer, it has the potential to be a world exporter of spices that supply positively to the economy³. One herb that is used as a medicinal plant is *Ocimum sanctum* L., also known as holy basil⁴. On the other hand, basil leaves (*Ocimum* spp.) have secondary metabolite and chemical compounds that can be used for insecticidal, larvicidal, nematocidal, antipyretic, fungicidal, antibacterial and antioxidant⁵. There are up to 160 species of basil that may exist⁶. However, *Ocimum sanctum* is one of the commercial basil known as herbal plants that have beneficial effects. Basil has some phytochemical and bioactive compounds such as essential oils, phenolic compounds, saponins, tannins, flavonoids, steroids, terpenoids, alkaloids, phenols, carbohydrates, lignin, starch and anthraquinone and these can be used as antibacterial⁷.

The use of basil leaf extract for antimicrobial has been reported by Kavyashree *et al.*⁴ that *Ocimum sanctum* also showed growth inhibition for *Klebsiella*, *E. coli*, *Staphylococcus aureus*, *Vibrio cholerae* and *Pseudomonas aeruginosa*. Dhale *et al.*⁷ reported that chloroform extract from basil leaves can inhibit the growth of *Shigella dysenteriae* bacteria and 100 mg mL⁻¹ of an alcohol extract from basil leaves can inhibit *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* with inhibitory zones 10, 12, 23 and 16 mm. Balakumar *et al.*⁸ reported *Ocimum sanctum* leaves showed effective antifungal activity against some of the fungi: *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum*, *M. nanum* and *Epidermophyton floccosum*.

In this study, basil leaf extract was used as the biological preservative against *E. coli* and *A. niger*. Different concentration of basil leaf extract was used to find the best concentration of basil leaf extract that can inhibit the growth of *E. coli* and *A. niger*.

MATERIALS AND METHODS

Study area: This research was conducted from December, 2021 to July, 2022 at the Food Microbiology Laboratory, Department of Food Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Bandung, Indonesia.

Materials: The basil leaves (*O. sanctum* L.) were obtained from Tugu Cihanjuang Village, Parongpong District, West Bandung Province, Indonesia. Flour, salt, oil, egg and water were used as ingredients for wet noodles. NA (Nutrient Agar), PDA (Potato Dextrose Agar), PCA (Plate Count Agar), LBSS (Lactose Broth Single Strength), LBDS (Lactose Broth Double Strength), EMB Agar (Eosin Methylene Blue) and NaCl 0.85%, 70% ethanol, 95% alcohol, *E. coli* and *A. niger* were used for microbial cultures.

Basil leaf extraction: The basil leaves were washed and dried in an oven at 50°C until dry for 6-7 days using sunlight to produce simplicia. Then, the sorting was conducted to separate unnecessary objects and other impurities that may have stuck during the drying process. The dried basil leaves were then reduced in size to enlarge the surface area and make the contact with the solvent more optimal. The dried leaves are then stored in an airtight container until further use⁸. Dried basil simplicia was then macerated using 70% ethanol and soaked for 2-3 days by stirring it every day. Then, it was filtered using a vacuum filter. The filtrate was obtained and condensed using a rotary evaporator with a heating bath temperature of 40°C and chiller at 3°C, for 1.5 hrs or depending on the amount of solution to obtain thick extract⁹.

Antimicrobial activity assay: Nutrient agar (NA) medium for *E. coli* growth media and potato dextrose agar (PDA) for *A. niger* growth media were prepared into a petri dish and allowed to freeze. Furthermore, 0.1 mL of *E. coli* suspension ($\lambda = 600$ nm) and 0.1 mL of suspension of *A. niger* ($\lambda = 530$ nm) were added and flattened throughout the surface of the media. Then, sterile disc paper was placed on the surface of the media and drops of basil leaf extract with various concentrations (0, 25, 50, 75 and 100%) up to 40 μ L. Incubation at 37°C for 1-2 days (*E. coli*) and temperature 25-28°C for 3-5 days (*A. niger*) to observe the inhibition zone. Finally, measurements of the diameter of the inhibition zone were formed using a calliper.

Determination of antimicrobial activity of basil: Making wet noodles was carried out based on the modification of Abidin *et al.*¹⁰ by mixing flour, salt, egg, soda ash, water and vegetable oil. A variety of treatments are made for the making

of wet noodles: Without any additions (control), add formalin (0.05%) and basil leaf extract (30% from the amount of water). Furthermore, the stirring of the mixture was conducted for 4-5 min and formed into sheets until the surface of it was flat and smooth. Then the dough was cut using a pasta maker as the desired length. The pieces of the mixture were then boiled in water (4 times the amount of flour) that has been added to coconut oil (10% boiled water) for +2 min until the cooked wet noodles were obtained.

Statistical analysis: All of the experiments in this research were carried out with 2 experiments with duplication and the results were expressed as Mean $\pm$ Standard Deviation (SD).

RESULTS

Determination of antimicrobial of basil leaf extract: Basil leaf extract inhibited *E. coli* growth (Fig. 1). The antibacterial activity of basil leaf extract started to be activated at 25% by forming an inhibition zone (19.08 mm), follows by 50, 75 and 100% of basil leaf extracts with the inhibition zone 21.01, 21.69 and 30.98 mm, respectively. The higher the concentration of basil leaf extract used, the higher the clear zone produced, which reached 30.98 mm at 100% of basil leaf extract. In the

contrast, the control did not show any activity against *E. coli*, indicated by the absence of visible inhibition zones around the disc paper.

The antimicrobial activity tended to increase at various concentrations of basil leaf extract against *A. niger* (Fig. 2). The control did not show any antimicrobial activity indicated by the absence of an inhibition zone around the disc paper. In contrast, the treatments of basil leaf extract effectively suppressed the growth of *A. niger*. The higher the concentration of basil leaf extract used, the bigger diameter of the inhibition zone obtained. The optimum concentration of basil leaf extract was obtained at 100% in which the diameter of the inhibition zone was 5.96 mm.

Quality testing of wet noodles: Basil leaf extract which has optimum inhibition against *E. coli* and *A. niger* is an extract with a concentration of 100%. This optimum concentration is then applied to wet noodles. The rapid growth of microbial was observed until 4 days after treatment in wet noodles treated with basil leaf extract and controlled during storage (Fig. 3). During observation of day 0, wet noodles added with basil leaf extract with a concentration of 100% had shown the total number of microbes that exceeded the standard (1×10^6 CFU g⁻¹), but 100% wet

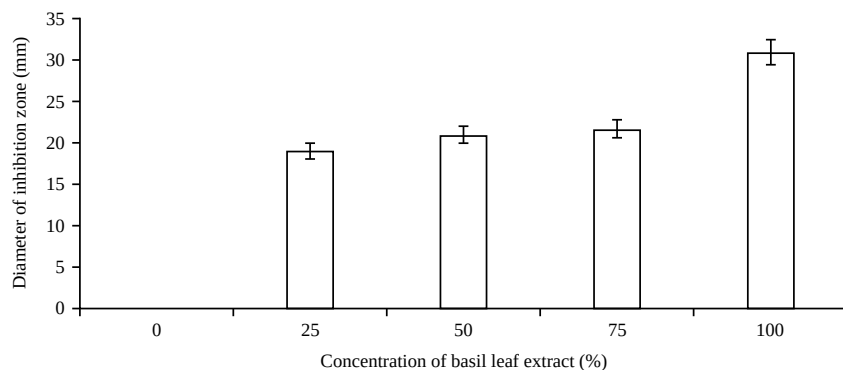


Fig. 1: Antimicrobial activity of basil leaf extract against *E. coli*

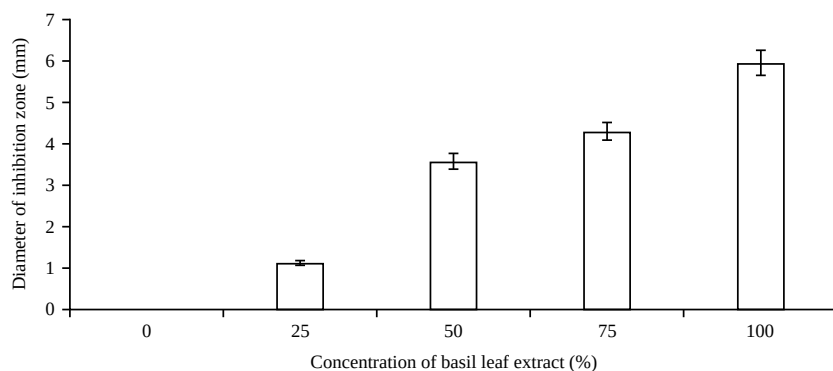


Fig. 2: Antimicrobial activity of basil leaf extract against *A. niger*

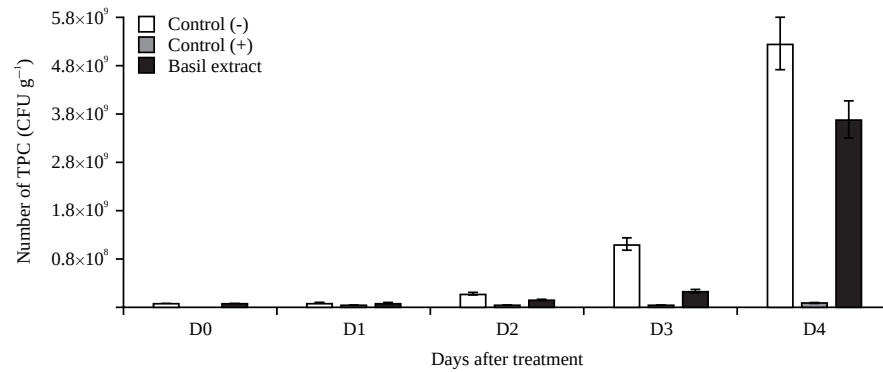


Fig. 3: Total plate count (TPC) in wet noodles at various treatments

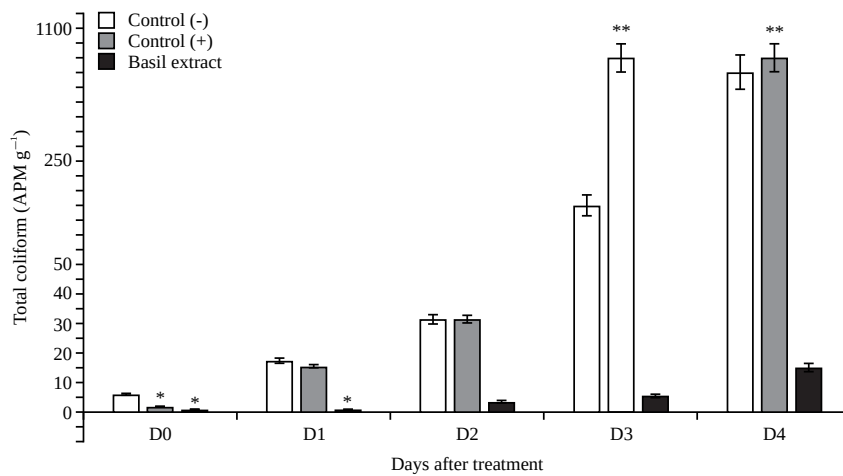


Fig. 4: Total coliform in wet noodles at various treatments

* < 3.6 APM/g and ** > 1100 APM/g

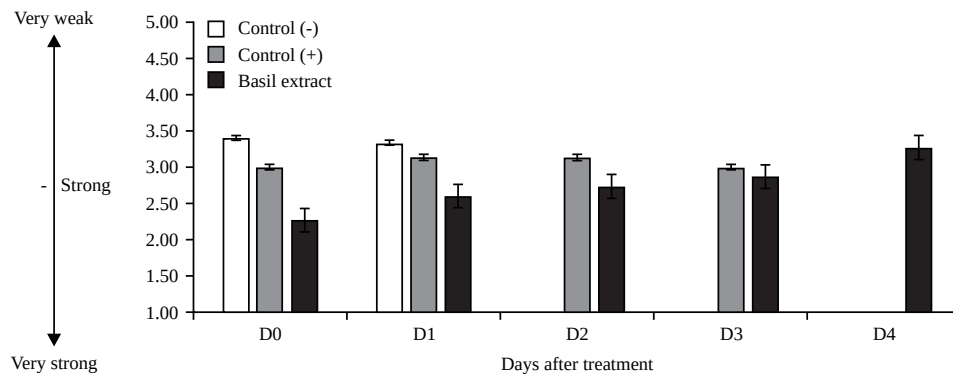


Fig. 5: Levels of preference fragrance for wet noodles at various treatments

basil extract of basil leaves still had physical characteristics such as colour and aroma that could be accepted. Two to four days after treatment, the number of TPC of wet noodles treated with basil leaf extract was lower than the control. However, the wet noodles treated with formalin had the lowest number of TPC in comparison with other treatments.

The wet noodles treated with basil leaf extract has the lowest total coliform until 4 days after treatment (Fig. 4), had the strongest aroma until 1 day after treatment (Fig. 5) and had good colour until 2 days after treatment (Fig. 6). However, the colour quality was reduced from 3-4 days after treatment. The wet noodles treated with formalin had the most elastic texture compared to other treatments (Fig. 7).

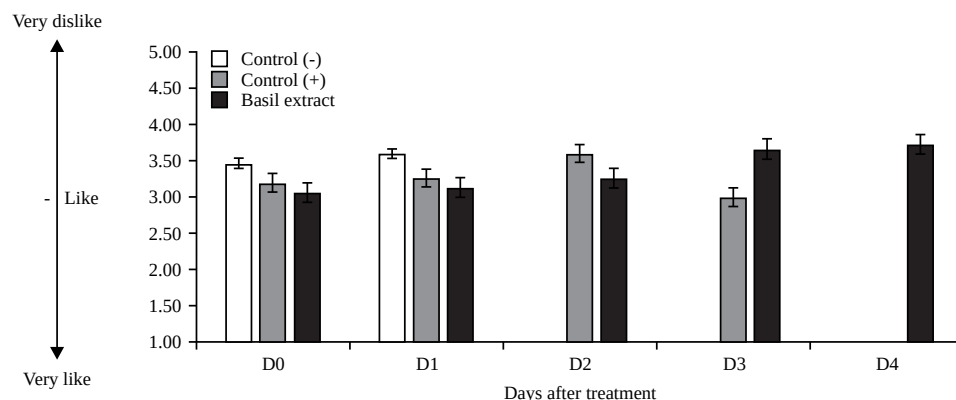


Fig. 6: Levels of preference color for wet noodles at various treatments

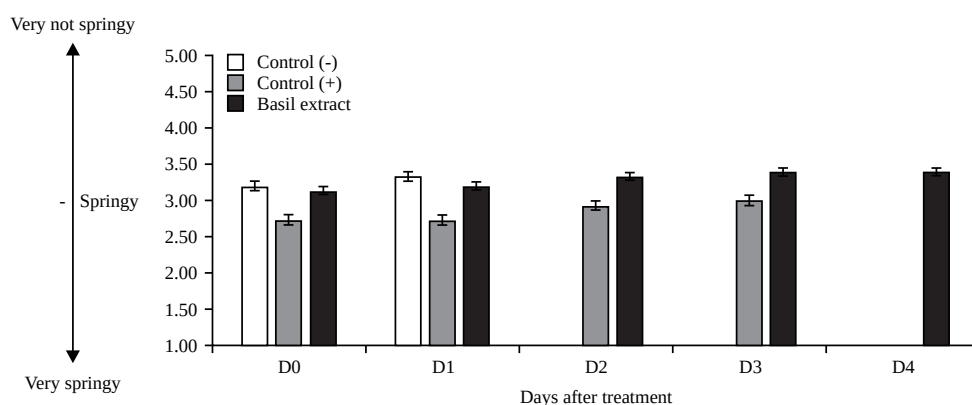


Fig. 7: Levels of preference texture for wet noodles at various treatments

DISCUSSION

These results suggested that the higher the concentration of basil leaf extract used, the greater the diameter of the inhibitory zone against *E. coli* and *A. niger* produced. Antimicrobial activity that inhibits the growth of *E. coli* and *A. niger* in basil leaf extract can be caused by the influence of the active compounds contained in the extract. Phytochemical compounds of basil leaves contained bioactive compounds such as aromatic compounds, rosmarinic acid, tannin, flavonoids and essential oils¹¹. In addition, solvent factors can also affect the bioactive compounds or secondary metabolites produced, the steroid group is soluble in non-polar solvents and tannin and flavonoid groups can be dissolved in polar solvents such as ethanol and alkaloid groups, in which they are insoluble in water¹². Moreover, the extract of *O. sanctum* contained flavonoids which are useful for antibacterial¹³. In addition, other bioactive compounds in basil leaf extract such as saponin, steroids and terpenoids. Secondary metabolites are also found and classified into metabolite groups including alcohol, amine, carboxylic acid, alkane, alkene, aldehyde,

phenol, ether, sulfur, halogen, benzene, nitrogen, sterol, amino acid, carbohydrate and nitrogen¹⁴.

The low diameter of the inhibition zone produced by basil leaf extract against *A. niger* may be caused by the ineffectiveness of bioactive compounds to suppress the growth of *A. niger*. As consequence, the bioactive compounds produced in this treatment were insufficient to produce high efficacy for inhibiting the growth of *A. niger*. In addition, antifungal activity may be affected by the essential oil contained in the basil leaf extract. The results were supported by El-Soud *et al.*¹⁵, antifungal activity of *O. basilicum* L., oil and its potential use as a pharmaceutical preservative in the treatment of fungal infections and against *A. flavus* growth and aflatoxin B1 production.

These results were similar to the previously reported result by Khalil¹⁶, in which the ethanol extract of basil leaf had antibacterial activity against *E. coli*. In addition, basil leaf extract has been reported to contain essential oil consisting of methyl chavicol, camphor, β -caryophyllene, eugenol, caryophyllene camphene and α -pinene which is useful for suppressing and preventing the growth of bacteria and fungi⁸,

which may reduce the quality of wet noodles. This result suggests that basil leaf extract produces antimicrobial activity due to the inhibitory zone suppressing the growth of *E. coli* (Fig. 1). Hossain *et al.*¹⁷ reported that essential oil and methanol extract of sweet basil *Ocimum basilicum* L. (Lamiaceae) had great antibacterial activity against food-borne pathogenic bacteria. Antibacterial activity against *E. coli* from essential oils from leaves, essential oils from stems and methanol extracts was 11.2, 12.2 and 14.1 mm, respectively.

The wet noodles treated with basil leaf extract had the strongest aroma until 1 day after treatment (Fig. 5). The strong aroma of wet noodles is treated with basil leaf extract because of its essential oil. This bioactive compound can inhibit microbial growth and prevent the sour aroma until 4 days after treatment. The control and formalin treatments were not observed until 4 days after treatment because the wet noodles treated with these treatments had a strong sour aroma and showed not good physical characteristics that were not suitable for consumption.

The wet noodles treated with basil leaf extract had good colour until 2 days after treatment (Fig. 6). However, the colour quality reduced from 3-4 days after treatment. The changes in food colours can be caused by several factors such as pigments, caramelization processes, Maillard reactions and the mixing of additional ingredients¹⁸. The chlorophyll content of basil leaf (*O. sanctum*) was low at the young stage (22 SPAD unit) and achieved its highest content at the mature stage (35 SPAD unit) where the leaf colour was light green to mature leaf with the highest chlorophyll content with the dark green of coloration¹⁹. In addition, the thickness and drought condition of leaves affected the degradation of chlorophyll content after harvest, the thicker the leaves, the harder to be degraded²⁰. These reasons may be the factor that affected the changes in brown colour in wet noodles treated with basil leaf extract.

The wet noodles treated with formalin had the most elastic texture compared to other treatments (Fig. 7). Formalin has an aldehyde element that easily reacts with protein. Therefore, the addition of formalin into food, such as tofu, would bind all proteins, degraded them and made the tofu more elastic.

Based on the results of this study, basil leaf extract can be considered a natural and safe preservative for wet noodles. The ability of basil leaf extracts to inhibit pathogenic microorganisms found in wet noodles was responsible for the high efficiency of basil leaf extracts in inhibiting bacterial growth²¹. The antibacterial effect of natural plant extracts is based on the ability of phenolic compounds contained in

natural extracts to disrupt cell membranes and induce oxidative stress leading to microbial lysis^{22,23}. The basil leaf extract can extend the shelf life by 4 days longer than the control and does not affect the sensory quality of taste and colour. Another study using basil extract as a natural preservative in fish products showed excellent results in inhibiting lipid oxidation and microbial degradation and improving sensory quality²⁴. Although this basil leaf extract was quite effective in inhibiting the growth of *E. coli* and extending shelf life, antibacterial testing against other pathogenic microorganisms should be performed to determine the effectiveness of basil leaf extract as a natural preservative in wet noodles and other food products. Furthermore, it is necessary to perform extraction methods other than maceration to determine the amount of bioactive compounds detected in the basil leaf extract.

CONCLUSION

The 70% ethanol phase basil leaf extract had strong antimicrobial activity at a concentration of 25% (19.08 mm) and was very strong at a concentration of 50% (21.01 mm), 75% (21.69 mm) and 100% (30.98 mm) against *E. coli*. Whereas, *A. niger* shows low antimicrobial activity in all concentrations used. The 100% extract concentration of basil leaf was chosen in the application of wet noodles because it produced the lowest total antimicrobial count on the 4th day.

SIGNIFICANCE STATEMENT

In this study, a suitable concentration of basil leaf extract was found. It can inhibit the growth of *E. coli* and *Aspergillus niger* to extend the shelf life of moist noodles. The active ingredients in basil leaf extract that are thought to have antibacterial properties are 2,6-octadiene-1,8-diol, exo-methylcamphenylol, camphor, phytol, linalool oxide, cis-geraniol, cis-lt's carveol. Investigating the use of basil leaves as a bio preservative for wet noodles has potential applications, as basil leaf extracts exhibited potent antibacterial activity against *E. coli* at all concentrations. In contrast, *A. niger* had low antibacterial activity. The results of this study will help the wet noodle industry adopt safer, healthier natural food preservatives.

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