



# Article Functional Characterization of Marigold Powder as a Food Ingredient for Lutein-Fortified Fresh Noodles

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**Abstract:** Marigold powder was utilized as a food ingredient to produce lutein-fortified fresh noodles for eye health, and its functionalities were characterized in terms of thermo-rheological, structural, and antioxidant properties. The pasting parameters and starch-gelatinization enthalpy values of wheat flour had a tendency to decrease with increasing levels of marigold powder. The use of marigold powder led to decreases in the storage and loss moduli of wheat flour pastes by weakening their cellular microstructure, which was confirmed by the scanning electron microscopic images. When marigold powder was incorporated into the formulation of fresh noodles, the cooking loss and water absorption of the noodles were not negatively affected at a level of 2% (w/w). Also, the noodles with 2% marigold powder were not significantly different from the control for the maximum resistance to extension. The levels of lutein in the noodles prepared with marigold powder (61.2 to 204.9 mg/100 g) were reduced by almost 50% after cooking. However, they seemed to satisfy the recommended daily dose of lutein for visual functions. Moreover, the use of marigold powder provided antioxidant properties for noodles by enhancing the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical-scavenging activities.

Keywords: marigold; lutein; noodle; rheology; structure

# 1. Introduction

Frequent exposure to various digital devices, such as smartphones and computers, may cause humans to experience digital eye strain (including blurred vision and eye fatigue), which is commonly linked to eye-related diseases [1]. In addition to the use of digital devices, aging [2] is recognized to be a primary factor increasing the risks to eye health. While age-related macular degeneration is a leading cause of blindness in the population over 65 years of age, the number of macular degeneration patients is steadily increasing every year [3]. Therefore, the global market of vision care is dramatically on the rise, and it is expected to reach approximately US \$74 billion by 2024 [4].

Health-conscious consumers have recently shifted the focus of the food industry to functional foods with beneficial health effects, since the bioactive compounds in foods are recognized to be very effective in reducing the risk of disease incidence and preventing illness [5]; the foods for eye health and eyesight are no exceptions. As a type of carotenoid, lutein is a major pigment in the macular region of the retina [6]. In addition, since lutein has an antioxidant effect that removes free radicals, it is recognized to prevent damage to the retinal cells [7]. Gale, et al. [8] reported that lutein played a significant role in promoting the health of the eyes, reducing the risk of age-related macular degeneration. It was also reported that lutein had great abilities to block blue light and to positively affect immune responses and inflammation [9]. However, since lutein cannot be synthesized by the body, it must be obtained from the diet [10]. It is known that lutein is available naturally in fruits,



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). cereals, and vegetables [11]. Marigold (*Tagetes erecta* L.) is also recognized to be a rich source of lutein. Thus, marigold powder has been commercially used as a nutritional supplement in capsules and tablets [12]. However, the applications of marigold powder (0.40–0.55%) to milk-based beverages whose physicochemical and sensory properties were characterized. The rheological changes of wheat flour by marigold powder were also reported [14]. However, there are no systematic studies about the processing performance of marigold powder high in lutein in a food system, which is typically subjected to a variety of processing conditions such as heat treatments and mixing with other ingredients. Consequently, the food industry may be discouraged to develop a variety of food products to maintain good eyesight and eye health.

In this study, marigold powder was incorporated as a source of lutein into the formulation of fresh noodles for lutein fortification, and its functionalities were investigated in terms of thermo-rheological, structural, and antioxidant properties.

### 2. Materials and Methods

#### 2.1. Materials

Marigold powder was obtained from Fell Nature Inc. (Gyeonggi-do, Korea), and its chemical composition consisted of 0.16% protein, 8.59% lipid, 0.21% ash, and 5.92% moisture, which were determined by the Association of Official Analytical Chemists (AOAC)-approved method. In addition, all-purpose wheat flour (CJ Co. Ltd., Seoul, Korea) was obtained from a commercial source. All the chemicals used in this study were of analytical grades.

#### 2.2. Pasting Property Measurement

The effect of marigold powder on the pasting pattern of wheat flour was evaluated using a starch pasting cell that was linked to a controlled-stress rheometer (Discovery HR-2 hybrid rheometer, TA instrument, New Castle, DE, USA). The wheat flour was replaced with marigold powder (0, 2, 4, and 6% w/w). Each flour sample (3 g) was suspended in in distilled water (25 g). The suspension was equilibrated at 50 °C for 1 min and then heated to 95 °C at a rate of 12 °C/min. This was followed by holding at 95 °C for 5 min, cooling to 50 °C at 12 °C/min, and maintained at 50 °C for 2 min.

#### 2.3. Thermal Analysis

The thermal properties of the wheat flour containing marigold powder at 0, 2, 4, and 6% by weight were investigated using differential scanning calorimetry (DSC) (DSC 200 F3 Maia, NETZSCH, Bavaria, Germany). The marigold powder–wheat flour mixture (5 mg) was held in an aluminum pan and distilled water (15  $\mu$ L) was added. After the pan was hermetically sealed, it was held at room temperature for 1 h for stabilization and then heated from 20 to 120 °C at 10 °C/min.

#### 2.4. Dynamic Viscoelastic Measurement

Right after the pasting measurement, the dynamic viscoelastic properties of the wheat flour–marigold powder pastes were investigated using a controlled-stress rheometer (Discovery HR-2 hybrid rheometer, TA instrument, New Castle DE, USA). A frequency sweep test was carried out at 30 °C in the frequency range from 0.1 to 10 Hz at a strain of 0.1%, which was within the linear viscoelastic regime.

#### 2.5. SEM Analysis

The effect of marigold powder on the microstructure of the wheat flour pastes was investigated using a scanning electron microscope (TM4000Plus, Hitachi High-Technologies, Tokyo, Japan). The wheat flour pastes where the wheat flour was replaced with marigold powder at 0, 2, 4, and 6% (w/w) were freeze-dried, and the microscope was operated at a magnification of ×1000 at a 15 kV accelerating voltage.

#### 2.6. Preparation of Fresh Noodles

The control of the fresh noodles was prepared with 50 g of wheat flour, 1 g NaCl (CJ Co.), and 20 mL of distilled water. The wheat flour was replaced with marigold powder (0, 2, 4, and 6% w/w). All the ingredients were mixed using a mixer (Kitchen Aid Inc., St. Joseph, MI, USA) at speed two for 3 min, and the dough sample was sheeted with a sheeting roller (1.4 mm), which was followed by cutting into noodle strands (4 mm wide and 50 mm long). The noodle strands (5 g) were cooked in hot distilled water (150 mL) for 3 and 6 min, and then drained in a strainer for 5 min.

#### 2.7. Cooking Property Measurement

The fresh noodle samples (5 g, 5 cm long) were immersed in 150 mL of boiling water for 3 and 6 min, followed by draining in a strainer for 5 min. The cooking water collected was dried at 105 °C for 6 h, and the residue was then weighed. The cooking loss and water absorption were determined based on the following equations:

Cooking loss (%) = weight of cooking water after drying  $\times$  100/weight of uncooked noodle

Water absorption (%) = (weight of cooked noodle – weight of uncooked noodle)  $\times$  100/weight of uncooked noodle

# 2.8. Tensile Measurement

The effect of marigold powder on the tensile properties of the noodles was investigated using a texture analyzer (TA-XT plus, Stable Micro System Ltd., Godalming, UK). After cooked for 6 min, the noodles were subjected to a tensile test using a Kieffer dough and gluten extensibility rig. While the noodle strand was extended at 200 mm/min, the maximum resistance force to extension (Rmax) and extensibility (E) were recorded from the curves of force versus distance.

# 2.9. HPLC Analysis of Lutein

According to the method of Liu et al. [15] and Moros et al. [16], with slight modifications, the level of lutein in marigold powder and noodles was quantitatively analyzed using high-performance liquid chromatography (HPLC, Ultimate 3000, Dionex, CA, USA) with a UV detector and a C30 column (CT99S05-2546WT, YMC Co. Ltd., Kyoto, Japan). For analyzing the level of lutein in the noodles, the noodles were freeze-dried and ground to pass through a 100-mesh sieve. The marigold or the noodle powder (0.1 g) was treated with 25 mL of 70% ethanol, and butylated-hydroxy-toluene (10 mg) was added to avoid the oxidation of the lutein. The sample was agitated at 50  $^{\circ}$ C for 4 h and centrifuged at  $4500 \times g$  (4 °C, 15 min). After the supernatant was collected, the residue was re-treated with 70% ethanol one more time. All the ethanolic extracts were combined and evaporated using a nitrogen evaporator (Organomation, Berlin, MA, USA) at 40 °C. The concentrated extracts were eluted with the use of two mobile phases that consisted of methanol/MTBE/water (81:15:4, v/v/v) (solvent A) and methanol/MTBE (9:91, v/v) (solvent B). After being dissolved in 2 mL of solvent A, the samples were filtered with 0.45 um syringe filters (Pall, New York, NY, USA). The linear gradient started from 100% solvent A and decreased to 50% within 45 min, followed by a second linear gradient to 100% solvent B within 15 min. The column temperature was set at 25  $^\circ$ C and the flow rate was 1.0 mL/min. External neat solvent calibration was performed by diluting suitable volumes of lutein standard (90.0%, Phytolab, Dutendorfer, Germany), and the calibration curve was shown to have a regression equation as follows:

$$y = 1.6972x + 0.3104$$
 (R2 = 0.9999).

#### 2.10. Antioxidant Activity Measurement

The changes in the antioxidant activities of the noodles by marigold powder were investigated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. The freeze-dried noodle samples were ground to pass

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through a 100-mesh sieve. The ground noodle powder was agitated with 70% ethanol at room temperature for 5 h, followed by centrifugation at  $15,000 \times g$  for 20 min. DPPH assay was conducted based on the method reported by Sun and Ho [17], with slight modifications. The ethanol extract (0.5 mL) was reacted with 0.1 mM DPPH solution (0.5 mL), followed by incubation at 37 °C for 30 min. The absorbance was recorded at 517 nm using a spectrophotometer (DU 730, Beckman Coulter Inc., Fullerton, CA, USA). In addition, the ABTS solution was prepared by the procedure of Bhattacharyya et al. [18]. The extract (0.1 mL) was added to the ABTS solution (1 mL), and the mixture was held at room temperature for 6 min. The absorbance was recorded at 734 nm. All the results were expressed as Trolox equivalent (TE) per gram of dry sample.

#### 2.11. Statistical Analysis

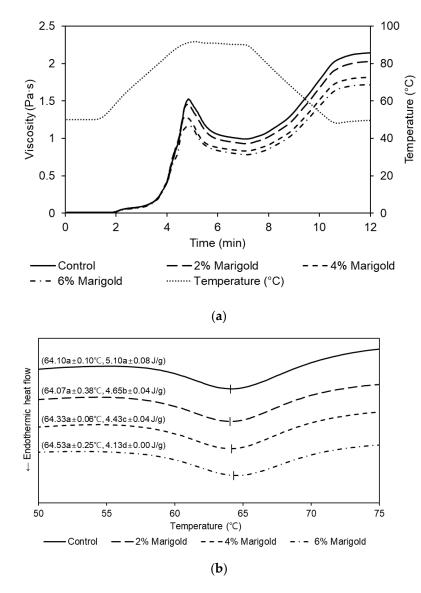
All the experiments were conducted in triplicate. The experimental results were analyzed using the R statistical package. The analysis of variance (ANOVA) was conducted at a confidence level of 95%, followed by Duncan's multiple range test for a mean comparison.

## 3. Results and Discussion

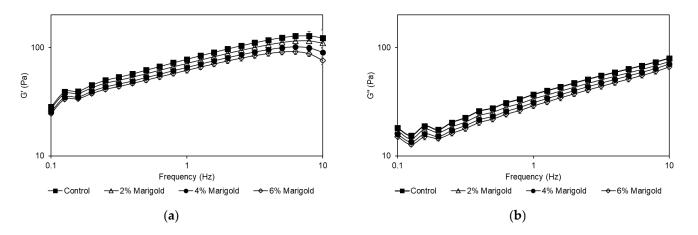
Figure 1a exhibits the pasting patterns of the wheat flour containing marigold powder. The pasting profiles of all the samples were distinctly characterized by peak viscosity during heating, a decrease in the viscosity during the temperature holding, and a viscosity increase during the cooling. This pasting pattern could be explained by the fact that starch granules in the wheat flour became swollen and ruptured during gelatinization and then recrystallized during retrogradation [19]. Therefore, as the wheat flour was replaced with marigold powder, the pasting parameters such as peak, through, and final viscosities became lower, which would be due mainly to the reduced level of starch granules derived from the wheat flour replacement with marigold powder. This pasting trend was also observed in preceding studies where wheat flour was replaced with corn bran [20] and  $\beta$ -glucan [21].

The effect of marigold powder on the thermal properties of the wheat flour was investigated as shown in Figure 1b. A distinct peak of starch gelatinization was clearly detected in the temperature range from 60 to 70 °C. The values of the peak enthalpy required to gelatinize the starch granules in the wheat flour tended to decrease with increasing amounts of marigold powder. However, as can be seen in Figure 1b, the use of marigold powder did not significantly influence the gelatinization temperature of the wheat flour (p > 0.05).

The changes in the dynamic viscoelastic properties of wheat flour paste due to marigold powder were examined. Figure 2 showed the storage (G') and loss (G") moduli of the paste samples, which are measures of the elastic and viscous nature of a material, respectively [22]. They had a tendency to increase when the frequency increased, showing frequency dependence. In addition, all the samples had higher values of storage moduli (G') than loss moduli (G"), which indicated a predominant elastic behavior. Thus, all of the samples exhibited weak-gel-like viscoelastic characteristics. It was also noted that the values of G' and G" decreased with increasing levels of marigold powder. These results showed that the replacement of the wheat flour with marigold powder weakened the structure of the paste samples, consequently contributing to the reduced viscoelastic parameters.



**Figure 1.** Effect of marigold powder on the pasting (**a**) and thermal (**b**) properties of wheat flour (means with different letters in the same column differ significantly at p < 0.05).



**Figure 2.** Changes in the viscoelastic properties of wheat flour pastes by marigold powder (G'(a) and G''(b)).

The microstructure of the starch paste samples with marigold powder was investigated using scanning electron microscopy. Figure 3 shows the three-dimensional structure of the paste samples with the different levels of marigold powder, exhibiting a cellular structure [23]. The paste sample without marigold powder appeared to have a dense and tight structure. However, the pores became larger in the paste samples with higher levels of marigold powder, probably weakening the structural network. Therefore, it appear that these structural changes with the addition of marigold powder were correlated with the reduced viscoelastic parameters as already mentioned in Figure 2.

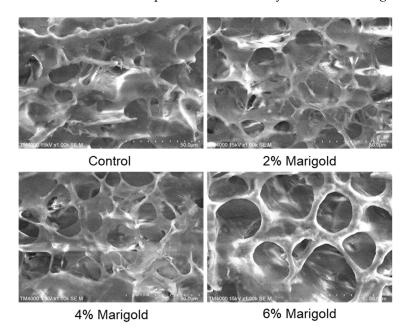
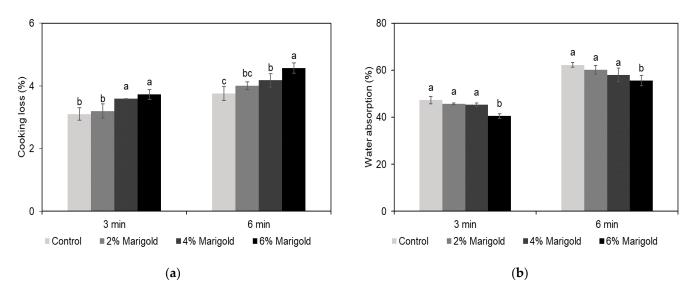


Figure 3. Microstructural changes of wheat flour pastes by marigold powder (×1000 magnification).

The cooking properties of the noodles prepared with marigold powder were investigated, and results are shown in Figure 4. It is well-recognized that solid components in noodles are released to cooking water when the noodles are cooked in hot water, consequently leading to a cooking loss [24]. Since a high cooking loss is related to a sticky noodle texture and viscous liquid, the cooking loss is recognized as one of important quality measures for noodles. Figure 4 shows the effect of marigold powder on the cooking properties of the noodles cooked for 3 and 6 min. The use of marigold powder at a level of 2% and 4% did not significantly affect the cooking loss and water absorption of the noodles after cooking, respectively. However, the samples prepared with a high level of marigold powder exhibited higher cooking loss and lower water absorption. The decreased water absorption of the noodles with marigold powder could be correlated with the reduced pasting parameters, specifically peak viscosity, which is known to be closed related to starch swelling volume [25].



**Figure 4.** Effect of marigold powder on the cooking loss (**a**) and water absorption (**b**) of fresh noodles (means with different letters on the bars differ significantly at p < 0.05).

These results were closely correlated with the structural characteristics of the noodles with marigold powder (see Figure 3).

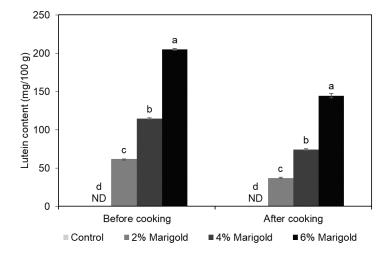
Table 1 shows the effect of marigold powder on the textural properties of marigoldpowder-incorporated wheat noodles, which were measured using a Kieffer dough and gluten extensibility rig. After cooking, the maximum resistance required to extend noodles (Rmax) and the distance at the maximum force (E) [26] were evaluated. The control sample without marigold powder had the highest value of Rmax (1.74 N) and E (19.14 mm), whereas the use of marigold powder for the wheat flour significantly reduced values of both Rmax and E. However, when marigold powder was incorporated at a level of 2% (w/w), there were no significant differences in the tensile properties (Rmax) from the control sample.

**Table 1.** Effect of marigold powder on the tensile properties of noodles after cooking (means with different letters in the same row differ significantly at p < 0.05).

	Control	2% Marigold	4% Marigold	6% Marigold
Rmax (N) E (mm)	$1.74 \pm 0.15$ <sup>a</sup> 19.14 $\pm$ 1.46 <sup>a</sup>	$1.66 \pm 0.08~^{a}$ $16.26 \pm 1.34~^{b}$	$1.27 \pm 0.08$ <sup>b</sup> $15.68 \pm 0.97$ <sup>b</sup>	$1.11 \pm 0.09\ ^{ m c}$ $15.06 \pm 0.79\ ^{ m b}$

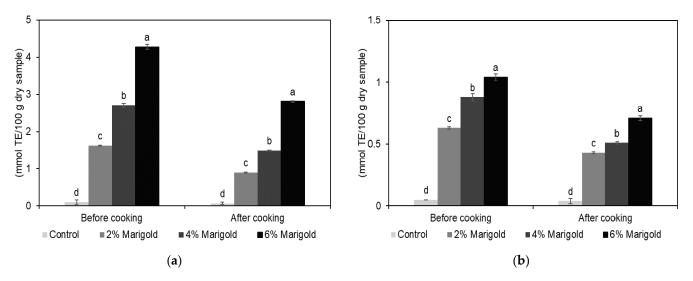
The HPLC analysis demonstrated that the level of lutein in the marigold powder was determined to be 2.639% (data not shown). This result could be favorably compared with the lutein contents of the different marigold cultivars in preceding studies, which were reported to be in the range of 0.831–2.059% for the petals [27] and 0.126–0.272 for the flowers [28]. The levels of lutein in the marigold-powder-incorporated fresh noodles were quantitatively analyzed before and after cooking using the HPLC. As shown in Figure 5, the contents of lutein in the noodles increased when the amounts of marigold powder were increased, whereas lutein was not detected in the control noodle sample made from wheat flour without marigold powder. Before cooking, the levels of lutein in the noodles prepared with marigold powder ranged from 61.2 to 204.9 mg/100 g noodle. After cooking, the levels of lutein in the noodles decreased to 37.1–144.6 mg/100 g noodle. Thus, the cooking process led to a distinct decrease in the content of lutein by almost 50%. This could be due to the heat labile feature of lutein, which was reported in preceding studies in which the level of lutein in whole grains [29] and vegetable oils [30] was reduced due to exposure to high temperatures. Furthermore, as mentioned in the cooking loss results (Figure 4a), the higher cooking loss of the noodles with marigold powder seemed to cause

lutein to be reduced. It is recommended to take 10 mg/day of lutein for improving visual functions [31]. Although the levels of lutein in the noodles were reduced after cooking, it appeared that they were enough to satisfy this recommendation.



**Figure 5.** The lutein contents of the noodles prepared with marigold powder before and after cooking (ND: not detected, means with different letters on the bars differ significantly at p < 0.05).

It is widely reported that marigold extracts have great abilities to scavenge radicals [32]. Thus, the effect of marigold powder on the antioxidant capacities of the noodles was examined before and after cooking. As shown in Figure 6, the antioxidant activities were expressed as Trolox equivalents. It was noted that the antioxidant activities of the noodles containing marigold powder were higher in both the DPPH (Figure 6a) and ABTS (Figure 6b) assays than those of the samples prepared without marigold powder. Furthermore, the scavenging activities had a tendency to increase when the amounts of marigold powder were increased. Thus, it was obvious that marigold powder positively contributed to enhancing the antioxidant capacities of the noodles. In addition, the antioxidant activities of the noodles were significantly reduced by almost 30% after cooking. This result could be explained by the fact that the cooking process led to the oxidative degradation of antioxidant compounds [33]. Also, as mentioned in Figure 4, the reduced antioxidant capacities might result from the leaching of the antioxidants from the noodles to the cooking water during cooking [34].



**Figure 6.** The antioxidant activities of the noodles prepared with marigold powder before and after cooking (DPPH (**a**) and ABTS (**b**)) (means with different letters on the bars differ significantly at p < 0.05).

# 4. Conclusions

Marigold powder was incorporated as a good source of lutein into the formulation of fresh noodles for lutein fortification, and its effects on the thermo-rheological, structural, and antioxidant properties of the noodles were characterized. The pasting and dynamic viscoelastic parameters of the wheat flour were reduced by increasing the levels of marigold powder, which weakened the cellular microstructure. However, the use of marigold powder at a level of 2% did not significantly affect the cooking and textural properties of the noodles. Moreover, the noodles prepared with marigold powder contained a high content of lutein (61.2–204.9 mg/100 g before cooking and 37.1–144.6 mg/100 g after cooking) that positively contributed to enhancing their antioxidant activities. Thus, this study may provide fundamental information related to the processing performance of marigold powder in a food system, which could give the food industry more potential opportunities to develop new functional food products for eye health using lutein fortification without significant quality loss. Further studies are necessary to extend the application of marigold powder as an excellent source of lutein to a wider variety of food products.

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