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Original article

Effect of nanocomposite-based packaging on preservation quality of green tea

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Summary An improved nanocomposite-based packaging material (NCP) was prepared by blending polyethylene with nano-Ag, nano-TiO_2 and attapulgite. And its effect on preservation quality of green tea (Biluochun and Qingfeng) during one-year ambient storage was investigated. The results showed that adding nanoparticles to the polyethylene could significantly improve the barrier property. After one-year ambient storage, the moisture of Biluochun and Qingfeng with NCP was decreased by 10.3% and 6.1% compared with the normal packaging material. Meanwhile, the contents of amino acid, tea polyphenols, chlorophyll and ascorbic acid were increased by 22.8%, 9.4%, 30.4% and 9.5% for Biluochun and 19.2%, 9.3%, 27.3% and 21.6% for Qingfeng, respectively, compared with the normal packaging material. Moreover, the sensory quality of NCP was also superior to that with the control. Therefore, the improved NCP material probably provides an attractive alternative to maintain the preservation quality of green tea at a high level during storage.

Keywords Green tea, nanocomposite-based packaging, preservation quality.

Introduction

Green tea, the favourite type consumed in Japan and China, has also become increasingly popular in Western countries in recent years because of its potential pharmacological properties such as antioxidant, antitumor and anticarcinogenic activities (Tomlins & Mashingaidze, 1997; Schut & Yao, 2000; Wang *et al.*, 2000). However, green tea quality is gradually decreased with oxidation of tea polyphenols, vitamin C and amino acid, decomposition of chlorophyll and browning during storage, which probably be ascribed to ambient moisture, oxygen and the residual enzymes. To minimise the effects of these factors on the preservation quality of green tea, various packaging materials have been applied to the storage of green tea, including a clear glass bottle, a wooden box (Wichremasinghe & Perera, 1972), a metal can, a polyethylene film, a paper/plastic laminate and plastic films (Fukatsu, 1978). However, many materials possess poor barrier property, weak mechanical property or undesirable preservation effects, which strongly limited their application for storage of green tea.

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Thus, there is an urgent need to have alternative technologies to inhibit the undesirable physicochemical and biochemical changes during storage. For this, the innovations in food packaging technology have been introduced with the increase in consumer demand. At present, the commonly used food packaging materials are still natural polymers, which are frequently blended with other synthetic polymers or, less frequently, chemically modified with the aim of extending their applications in more special or severe circumstances (Guilbert *et al.*, 1997; Petersen *et al.*, 1999; Charton *et al.*, 2006). Although the application of biodegradable films for food packaging has been employed, the poor barrier properties and weak mechanical properties strongly limited its further application in food storage.

In recent years, nanomaterials have attracted increasing attention because of their unique advantages. Compared with the traditional materials, nanophase materials exhibit unusual chemical, mechanical, optical, electrical and magnetic properties (Li *et al.*, 2002; El Naschie, 2006). Nano-TiO_2 with light catalysing

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capability could oxidise ethylene into water and CO_2 (Poddar *et al.*, 2004). Nanosilver with little dimension effect, quanta effect and large external area effect caused the more effective antibacterial activity than Ag^+. Moreover, the nanotechnology has also been widely applied to food storage. Fuji apples with nano-SiOx/chitosan keeping fresh agents had higher preservation quality than control according to the research described by Li & Wang (2006). Researchers from Chinese Academy of Science successfully prepared nanoplastics NCPET bottle, which could extend the shelf life of beer to 4–5 months and almost keep the same level as fresh beer (He, 2001). The application of nanocomposite concept has been proven to be a promising option to improve mechanical and barrier properties (Avella *et al.*, 2005). In our previous study, the nanopackaging material had a quite beneficial effect on physicochemical and sensory quality of Chinese jujube compared with normal packaging material during the 12-day storage (Li *et al.*, 2009). Further study indicated that this nanopackaging material possesses better barrier and mechanical properties. Its water vapour permeability was decreased by 28.0%, while its longitudinal strength was 1.24-fold higher than the polyethylene films. To the best of our knowledge, there seems to be few investigations focusing on the effect of nanocomposite-based packaging on preservation of green tea. Therefore, as a part of our ongoing study, we improved the formula of nanocomposite-based packaging materials prepared previously in our laboratory, aiming to develop a preferable nanocomposite-based packaging material to preserve green tea.. To well understand the effect of this improved nanocomposite-based packaging material, the morphological characterisation, barrier properties and its effect on the contents of amino acid, tea polyphenols, chlorophyll and ascorbic acid were investigated in this study.

Materials and methods

Materials

Nanocomposite-based packaging material

Nanopowder (attapulgite:Ag3PO4:anatase TiO2:rutile TiO2 = 5:2:4:1, 30%), polyethylene (56%) and cross-link reagent KH-570 (14%) were immingled to uniformity for 0.5 h through a high-speed mixer, extruded out by a twin screw extruder and then cut into nanogranules after cooling for 2 min. Subsequently, 30 kg polyethylene granule and 5 kg mixed nanogranule were immingled for 0.5 h and then blown into films of 65 μm thickness. After cooling, another aluminium film of 15 μm thickness was stuck on the above films to form the nanocomposite-based packaging material (Li *et al.*, 2002). Finally, the combined film was made into bags of 30×20 cm using a bag-making machine (FBD-300W; JiaQi Packaging Machiner Co., Zhejiang, China). The normal packaging material without nanopowder masterbatch was produced by sticking the same aluminium film to the polyethylene films of the same thickness as NCP, and the final thickness of two films was 80 μm and made into the bags of the same size, which was served as control.

Preparation of green tea

Two kinds of commercial green tea Biluochun and Qingfeng were obtained from Fukun Tea Co., Yixing, Jiangsu province, China. One bud and two leaves of the first flush shoots were plucked on 20 April and processed using the commercial processing technology. Immediately, the tea leaves were packed separately using the nanocomposite-based packaging material (NCP) bags and normal polyethylene bags (~50 g per pack). All packets were stored at ambient conditions (20 ± 2 °C; 90% RH) as a high temperature and high humidity model, then withdrawn at two-month interval for the respective analysis. Before analysis, the green tea leaves were milled into fine powder using a pulverizer (Philips HR2864, Zhuhai, China).

Methods

Physical property analysis and microstructure observation of packaging material

According to China National Analysis Standards (GB/T1037 1988, GB/T1038 2000), the physical property of packaging materials, including water vapour permeability and oxygen permeability, was measured by the sheet-cup method and differential pressure method, respectively. The test film was sealed to a permeation cell with a 50% RH gradient across the film at 23 °C. Water vapour permeability and oxygen permeability were analysed using TSY-T1 water vapour permeability tester and BTY-B1 oxygen permeability tester (Shanghai Huayan Instrument & Equipment Co. Ltd., Shanghai, China), respectively. The microstructure of materials was observed using a scanning electron microscope (S-3000N, Hitachi High-Technologies Co., Tokyo, Japan) at an accelerating voltage of 20 kV.

Determination of moisture content

Moisture content of the green tea samples was monitored using the method recommended by ISO (1980). Samples were dried at 103 ± 2 °C for 6 h prior to determination, and moisture content was expressed in percentage (Thomas *et al.*, 2008).

Determination of amino acids

Three grams of powdered tea samples was infused with 450 mL freshly boiled distilled water and then incubated in a boiling water bath for 45 min with general shaking at every 10-min interval. The tea infusion was filtrated using double-layer filter papers, and the volume was fixed to 500 mL after fully cooling down as stock solution for further analysis.

One millilitre of stock tea infusion, 0.5 mL phosphate buffer (pH 8.0) and 0.5 mL of 2% ninhydrin solution were mixed in a 25-mL volumetric flask and then heated at 100 °C for 15 min. After cooling, the flask was completed to 25 mL with distilled water. The OD_{570} was measured using a JH-722 visible spectrophotometer (Shimadzu Corporation, Tokyo, Japan). The content of amino acids was calculated according to the formula below:

$$\text{Aminoacids}\left(\text{g kg}^{-1}\right) = \frac{CL_1/1000L_2}{Mm} \times 1000$$

where L_1 was the total volume of the tea infusion (mL), L_2 was the volume of infusion taken to reaction (mL), M was the dry weight of the tea sample (g), m was the dry ratio of the tea sample and C was the mass of glutamic acid (mg/OD_{570}) obtained according to the OD_{570} from a standard curve with glutamic acid as a standard component (Chen & Zhou, 2005).

Determination of tea polyphenols

One millilitre of stock tea infusion, 4 mL distilled water and 5 mL reaction solution containing 0.1% ferrous sulphate and 0.5% potassium sodium tartrate were mixed and fixed to 25 mL with PBS (pH 7.5). The OD_{540} was measured using a 10-mm colour comparison cell in a spectrophotometer (JH-722Vis; Shimadzu Corporation). The tea polyphenol content was calculated using the formula:

$$\text{Teapolyphenols}\left(\text{g kg}^{-1}\right) = \frac{OD_{540} \times 3.914}{100} \times \frac{L_1 \times 100}{L_2 Mm}$$

where L_1 was the total volume of the tea infusion (mL), L_2 was the volume of infusion taken to reaction (mL), M was the dry weight of tea sample (g), m was the dry ratio of the tea sample and 3.914 corresponded that 1 OD_{540} was equal to 3.914 mg tea polyphenols in the tea infusion (Chen & Zhou, 2005).

Determination of chlorophyll

Approximately 0.1 g tea powder was extracted by 50 mL of 80% (v/v) acetone for 2 min and then filtered. The extract was determined spectrophotometrically at the wavelength of 663 nm (chlorophyll a) and 645 nm (chlorophyll b), respectively. The chlorophyll content was calculated using the formula below (Huang *et al.*, 2007):

$$C_a\ (\text{mg L}^{-1}) = 12.7\ \text{OD}\ 663 - 2.95\ \text{OD}\ 645,$$
$$C_b(\text{mg L}^{-1}) = 22.9\ \text{OD}\ 645 - 4.67\ \text{OD}\ 663,$$
$$C_t\ (\text{mg L}^{-1}) = C_a + C_b,$$

$$\text{Chlorophyll}\left(\text{g kg}^{-1}\right) = \frac{C_t \times 50}{M \times 1000}$$

where C_a was the concentration of chlorophyll a (mg L^{-1}), C_b was the concentration of chlorophyll b (mg L^{-1}), C_t was the total concentration of chlorophyll (mg L^{-1}) and M was the dry weight of tea sample (g).

Determination of ascorbic acid

The ascorbic acid of green tea was measured by 2, 6-dichlorophenolindophenol titration. Briefly, about 1.0 g tea powder was extracted in 50 mL of 1% oxalic acid and then centrifuged at 15 000 ***g*** and 4 °C for 15 min. After that, 5 mL of supernatant was titrated to a permanent pink colour by 0.025% 2,6-dichloropheno-lindophenol titration. Ascorbic acid concentration was calculated according to the titration volume of 2, 6-dichlorophenolindophenol and expressed as g kg^{-1} dry weight (Lu & Tan, 1994).

Sensory evaluation

Sensory quality of green tea was assessed according to the previous methods (Hu *et al.*, 2001; Xia *et al.*, 2006). Aqueous extraction of tea leaves was carried out under controlled conditions, so as to give the extracts the optimum liquor colour, aroma, taste and infused leaf characteristics. Three grams of tea was randomly chosen from each packaging and extracted with 100 mL boiling distilled water for 4 min and then filtered to collect the supernatant for sensory evaluation in Food Analysis Laboratory of Nanjing Agricultural University. Three 40-min sessions were conducted. Panellists, sitting around table, were provided with a broad selection of reference samples (five tea samples plus a commercial tea) representing the quality range of our products. Ten trained panellists were selected from twenty volunteers to undertake a sensory evaluation, aiming at the colour, liquor colour, aroma, taste and infused leaf. The panellists evaluated the samples individually and recorded the intensities for each index using 0–100 intensity scale, where 0 means 'none' and 100 means 'the highest intensity'. The total score of sensory quality was calculated on the basis of weights of various attributes, of which colour, liquor colour, aroma, taste and infused leaf accounted for 30%, 10%, 25%, 25% and 10%, respectively (Owuor & Benjamin, 2003).

Statistical analysis

All analyses were carried out in triplicate, and data were expressed as mean ± SD. A one-way analysis of variance (ANOVA) was performed to calculate significant differences in treatment means, and a probability value of <0.05 was considered significant. All computations were made by employing the statistical software SAS (version 8.0).

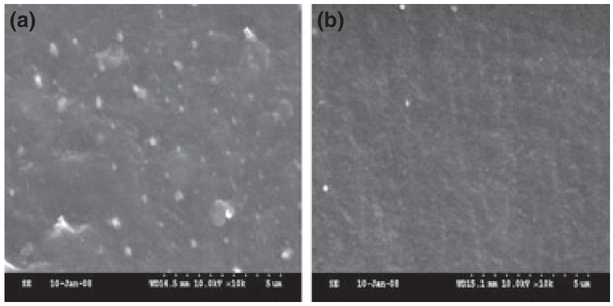

Figure 1 SEM micrographs of nanocomposite-based packaging material (NCP) (a) and normal packing materials (b).

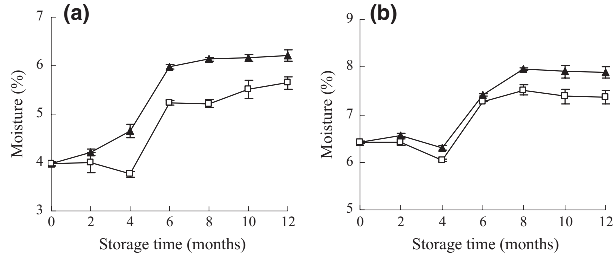

Figure 2 Effect of nanocomposite-based packaging material (NCP) and normal packing on moisture content of green tea during storage. (a) Biluochun tea; (b) Qingfeng tea; -▲-, Normal packing; -□-, NCP.

Results and discussion

Physical properties and microstructure of packaging materials

As to the microstructure observation, the nanoparticles (attapulgite, Ag_3PO_4, anatase-TiO_2 and rutile-TiO_2) uniformly distributed in the NCP film with irregular shape (Fig. 1a). The dimension of most of these nanoparticles was in the range of 100–300 nm. Meanwhile, a few particles were found in normal packaging material (Fig. 1b). The water vapour and oxygen permeability of NCP material were 1.03 g m^{-2}·24 h and 0.29 cm^3 m^{-2}·24 h, respectively, which were decreased by 44.3% and 44.6% compared with the normal packaging material. These results indicated the improved NCP material had higher barrier property to H_2O and O_2 than the NCP material (2.05 g m^{-2}·24 h and 12.56 cm^3 m^{-2}·24 h for water vapour and oxygen permeability, respectively) we prepared in our previous study (Li *et al.*, 2009). Ag, TiO_2 and kaolin were presented as nanoparticles in the previous NCP material, which expand Chinese jujube shelf life. However, attapulgite was selected instead of kaolin as well as optimal proportion to improve this nanocomposite-based material in the present study. Attapulgite is a kind of clay, which could obstruct small molecules like O_2 and H_2O. The decrease in green tea quality during a long-term storage is probably ascribed to ambient moisture, oxygen and the residual enzymes activated. In general, the higher barrier properties of NCP material might be attributed to the presence of nanoattapulgite we prepared. Similar results have been reported in recent years. Intercalated PS/org-clay nanocomposites showed more improvement for O_2 permeability (Meneghetti & Qutubuddin, 2006; Durmus *et al.*, 2007). Some of the clay layers in the intercalated sample formed a staircase-like arrangement, which created a more tortuous path for the gas diffusing molecule to traverse the nanocomposite film.

Effect of NCP on moisture content

Tea, being hygroscopic, was more susceptible to lipid hydrolysis, auto-oxidative reactions and enzymatic browning during storage, which were found to be accelerated by moisture (Okinda Owuor *et al.*, 2006). Increase in the moisture content over a period of time resulted in the deterioration of quality constituents of tea, which was characterised by a loss of aroma and astringency and sometimes by the acquisition of undesirable taints that caused the loss of quality constituents. The moisture content of NCP was always lower than the normal packaging material during storage in both Biluochun and Qingfeng (Fig. 2), which might be due to the higher barrier property of the NCP material to H_2O.

Effect of NCP on content of amino acids

Amino acid, a contributor to the taste and colour, was considered to be one of the significant parameters for tea quality assurance (Harbowy, 1997; Yao *et al.*, 2006). It has been demonstrated that there is a good positive correlation between green tea quality and content of amino acid (Chu *et al.*, 1997). The amino acid content during storage in Biluochun and Qingfeng was given in Fig. 3a,b. It varied complicatedly and showed a wavy trend during 12-month storage. Generally, the amino acid content of NCP was always higher than the normal packaging material. The difference was even more distinct during the late stages of the storage. NCP retained the amino acid content at 10.9 and 11.5 g kg^{-1} of Biluochun and Qingfeng after 12-month storage, which significantly increased by 25.3% and 21.1% compared with normal packaging material ($P < 0.05$). Some studies have showed that amino acid content was correlated with its oxidation and protein hydrolysis. Oxidation process decreased the level of amino acid during storage, while the hydrolysation of some soluble protein or the releases of some embedded amino acid increased amino acid content (Zhao *et al.*, 2008). It appeared that higher barrier property of NCP to O_2 and H_2O is

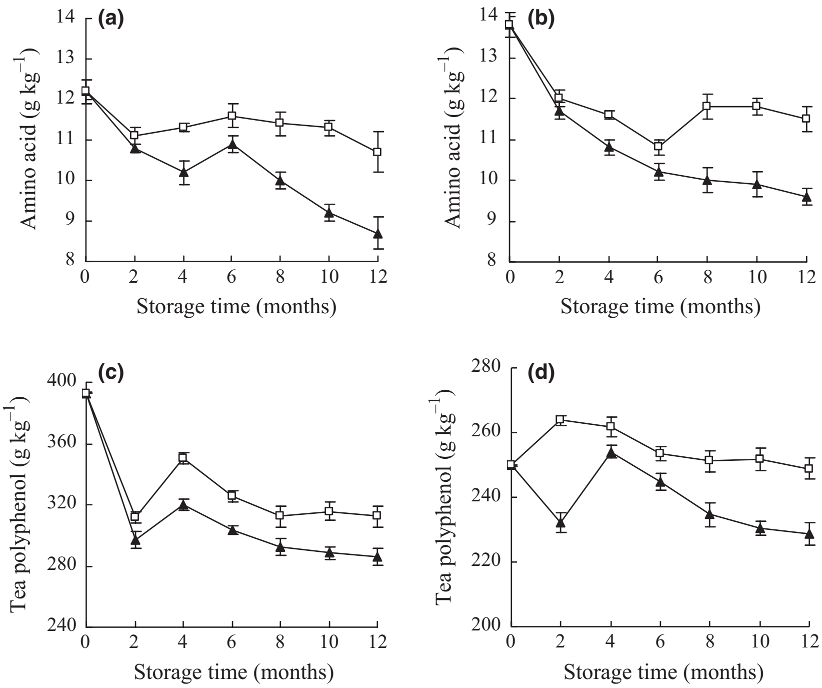

Figure 3 Effect of nanocomposite-based packaging material (NCP) and normal packing on amino acids and tea polyphenol contents of green tea during storage. (a, c) Biluochun tea; (b, d) Qingfeng tea; -▲-, Normal packing; -□-, NCP.

favourable to maintain a high level of amino acid of green tea.

Effect of NCP on content of tea polyphenols

As illustrated in Fig. 3c,d, the tea polyphenol content decreased continuously for both packaging except slightly increased around 4 months, which might be caused by transforming of small amount of insoluble phenols to soluble phenols (Cui *et al*., 2008). Generally, the tea polyphenol content of NCP group was always higher, and the changing trend was also gentler than the normal packaging material. Sang *et al*. (2005) reported that oxygen concentrations would influence polyphenol stability. Higher oxygen levels could accelerate catechin oxidation. Under lower O_2 concentration (flushed with N_2) at 37 °C, EGCG remained stable with only 5% degradation after 6 h. Corey *et al*. (2011) showed that polyphenols degraded more rapidly at a higher moisture environment and storage at Aw of 0.75 had the highest impact on polyphenol stability. Our result indicated that NCP could keep the content of tea polyphenol at a higher level compared with normal packaging material, which might be attributed to its higher barrier property to O_2 and H_2O.

Effect of NCP on content of chlorophyll

Chlorophyll, the main component of the colour in green tea, decomposed easily during storage and become unfavourable to the colour. NCP samples retained the chlorophyll level at 0.89 g kg^{-1} for Biluochun (Fig. 4a) and 1.06 g kg^{-1} for Qingfeng (Fig. 4b), respectively, which were more than 36.9% and 34.2% compared with normal packaging material after 12-month storage (P < 0.05). The transformation of chlorophyll had an important relation with the removal of magnesium. Both the increase in moisture and temperature could accelerate the magnesium removal (Huo & Jiang, 1987). Thus, the higher barrier property of NCP material to O_2 maintained the more chlorophyll content.

Effect of NCP on content of ascorbic acid

Although ascorbic acid has some protective effect on the quality of green tea, it could be easily oxidised, especially in high humidity and oxygen. As shown in Fig. 4c,d, the ascorbic acid decreased continuously for both packaging throughout the storage. However, NCP showed a better maintenance of ascorbic acid than that of the normal packaging material. Our previous research showed that ascorbic acid content of Chinese jujube was significantly higher in nanopackaging groups than in the corresponding controls (Li *et al*., 2009). This was also proved during the storage of fresh strawberry with nanopackaging material (Yang *et al*., 2010). Ayranci & Tunc (2003) thought oxygen is responsible for vitamin loss of many fruits and vegetables. Lower oxygen atmospheres can slow ascorbic acid loss by inhibiting its oxidation. Therefore, the degradation of ascorbic acid of green tea was lower in NCP than that in normal packaging, which might be attributed to low oxygen

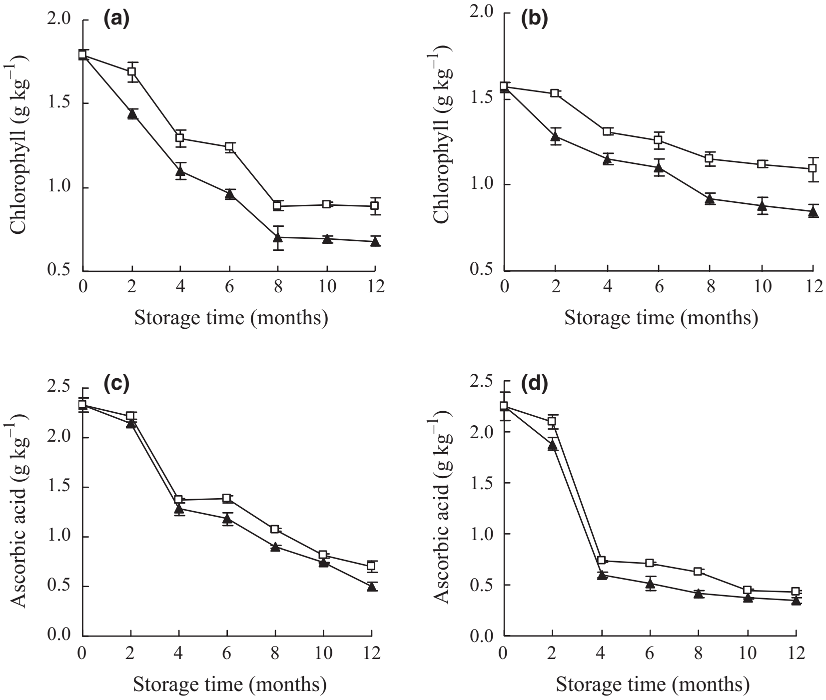

Figure 4 Effect of nanocomposite-based packaging material (NCP) and normal packing on chlorophyll and ascorbic acid contents of green tea during storage. (a, c) Biluochun tea; (b, d) Qingfeng tea; -▲-, Normal packing; -□-, NCP.

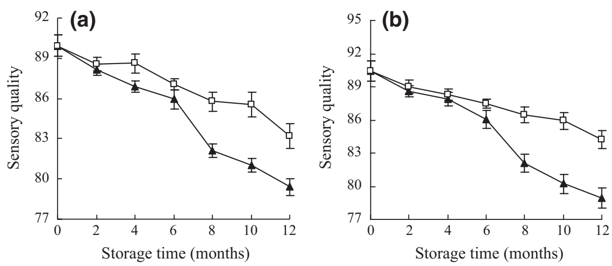

Figure 5 Effect of nanocomposite-based packaging material (NCP) and normal packing on sensory quality of green tea during storage. (a) Biluochun tea; (b) Qingfeng tea; -▲-, Normal packing; -□-, NCP.

environment formed by NCP with a preferable gas barrier property.

Effect of NCP on sensory quality

Sensory evaluation of green teas in NCP and normal packaging material were performed during 12-month storage. As shown in Fig. 5, the total score of sensory quality of both Biluochun and Qingfeng in NCP was always higher than that in the normal packaging group during the storage. The longer the storage, the higher the difference in the sensory quality was observed. The results on sensory evaluation were in agreement with the analysis of physicochemical indices. The physicochemical attributes play a decisive role in sensory quality of green tea. Many factors may change the sensory quality (i.e. colour, aroma, taste), including humidity, temperature, light and so on. The green tea samples packaged with NCP could block most of these factors except temperature to a certain extent.

Conclusions

An improved NCP material with lower water vapour and oxygen permeability was successfully synthesised and then was applied to the preservation of green tea according to its quality decrease during a long-term storage. Results showed that this improved NCP material had quite beneficial effect on physicochemical and sensory quality compared with normal packaging material. Furthermore, the NCP materials had the advantages of simple processing and feasibility to be industrialised in contrast to other storages, some of which are time-consuming, high-cost, colour changing and off flavour altering. Therefore, the nanocomposite-based packaging may provide an attractive alternative to improve the preservation quality of commercial tea product at a high level during extended storage.

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