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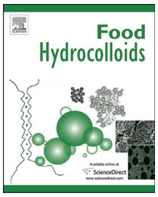

A new methodology combining microscopy observation with Artificial Neural Networks for the study of starch gelatinization

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ABSTRACT

A novel methodology combining microscopy observation with Artificial Neural Networks (ANNs) and realized by machine learning algorithms for the study of starch gelatinization was developed. As the most critical part during object detection, an improved starch single shot multi-box detector (starch-SSD) originated from ANNs was purposely designed and applied in monitoring the morphological changes of starch with increasing temperature. In the case, the birefringences were automatically identified by computer vision and then the relative birefringence number of the image was calculated. Basing on such number change, the temperature of phase transition was detected and consequently the degree of gelatinization (DG) at specific temperature was quickly calculated. Compared with traditional methods that mainly performed by manual operation, experimental results confirmed that the proposed method has competitive accuracy and is much faster. It also provides a unified standard for microscopy observation without subjective uncertainty.

1. Introduction

Starch is one of the most common biomacromolecules present in nature, and usually consists of two main components: linear amylose and highly branched amylopectin. Native starches significantly contribute to texture properties of starchy system and are widely applied in food and industrial applications. Generally, most of these properties are triggered when starch is heated in the presence of water. In the course, starch granules gradually swell and finally lose their crystallinity and molecular organization, which is well defined as gelatinization and is one of the vital important processes contributing to the industrial application of starch (Parker & Ring, 2001; Zobel, 1988).

Differential scanning calorimetry (DSC) and optical microscopy observation are technologies commonly used for the investigation of starch phase transition and associated properties in situ. These two methods measure different physicochemical properties and have unique and inherent advantages and disadvantages. Since Stevens Stevens, Elton, and Albnas (1971) first reported the application of DSC to study the thermal behaviors of starch gelatinization, DSC has been regarded as one of the most suitable methods for quantifying the endothermic behavior during gelatinization, covering both the range of gelatinization temperature and the energy requirements (Cooke & Gidley, 1992; Liu, Yu, Xie, & Chen, 2006; Tester & Morris, 1990). However, a number of report results obtained by DSC measurements are not consistent and sometimes controversial because of the complexity of the thermal behavior of starch and different experimental operations such as sample preparation, type of pan and measurement conditions (Yu & Christie, 2001). There are up to 4 endotherms evidently observed in the gelatinization process of normal starch, which are mainly dependent on water content and starch type (Biliaderis, Page, Slade, & Sirett, 1985; Liu et al., 2006; Russell, 1987; Shogren, 1992; Tester & Morris, 1990). However, in this system

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less than 90% water content are suggested since higher moisture will significantly decrease sensitivity of the equipment and sometimes even induced huge pressure to break the container during heating process (Liu et al., 2006; Yu & Christie, 2001). Based on the relative DSC studies, several theories have been proposed in order to explain the thermal mechanism, however, no model has generally or universally been accepted until now.

Polarized light microscopy with a hot-stage, which is able to directly observe simultaneous granular destruction and the disappearance of birefringence using both normal and polarized light, also can be applied to study starch gelatinization in situ and in real time under controlled heating conditions (Molina, Leiva, & Bouchon, 2016; Yeh & Li, 1996; Zhao et al., 2015). In case studies of pea and potato starch, it was stated that the disruption of starch structure started from the hilum area, propagated along the granule and accompanied by swelling of disrupted areas (Bogracheva, Meares, & Hedley, 2006; Tahir, Ellis, Bogracheva, Mearestaylor, & Butterworth, 2011). As the temperature rises to a critical value, the birefringences of the starch granules lost (Li, Li, Li, & Tatsumi, 2004). For a population of granules, the range for gelatinization temperature is usually between 5 and 10 °C, meaning that fractions of granules exhibit different gelatinization temperatures (Lund & Lorenz, 1984). When Spies and Hoseney (1982) firstly studied starch gelatinization using a Koefler hot-stage microscope, they referred to the temperature at which 50% of the granules had lost birefringence as gelatinization temperature (Spies & Hoseney, 1982). Later, Bryant and Hamaker (1997) designated the temperature at which the first granule was noted to lose birefringence as the onset temperature, and that at which 95% of the granules had lost birefringence as the gelatinization temperature end-point (Bryant & Hamaker, 1997), whereas others as that at which all (or 98%) granules lost birefringence as end-point (Chen, Yu, Kealy, Chen, & Li, 2007; Lund & Lorenz, 1984). In fact, there is no unified standard used to determine the transition temperature during gelatinization. Moreover, the traditional estimation of the change of birefringence relies on manual assessment by trained workers, which is time-consuming, tedious and subjective. Therefore, the results were reported subjectively by different researchers and shown that the visual judgment of gelatinization temperature by either criterion varies greatly from one observer to another, which caused some inconsistent and even confused conclusions. In the past years, though a few methods have been positively designed to evaluate the process of starch gelatinization with the changes of optical density of birefringence (Li, Xie, Yu, & Gao, 2014; Zhao et al., 2015) and granule size (Chen et al., 2007; Yeh & Li, 1996) through image analyzing techniques, such visual observations were also marked by subjective uncertainties, and long and costly litigation over discovery.

Artificial Neural Networks (ANNs), being a subfield of computer science, is classified as an important way to achieve artificial intelligence. It can be used in several domains and its advantage lies in the fact that the model can solve problems without being explicitly programmed. These models could easily reveal the implicit relations between inputs and outputs even if the representation is explicitly impossible, and such characteristic allows the use of machine learning models in many cases, for example in pattern recognition, classification problems (Voyant et al., 2017). Also as a crucial branch of machine learning, ANNs have brought huge improvements in object detection in resolving the most studied problems of visual recognition tasks, being used in several fields such as multiagent domain and biology (Descombes, 2017; Silveret al., 2016). In these studies, some algorithms such as adaptive image segmentation based on contour tracking, nonlinear arctan function transform were adopted to improve image quality and achieve effective inspection results. However, to the best of our knowledge, there are few studies performed using such achievements in food engineering (Lu, 2013), especially in the field of starch science.

The main focus of the present research is to propose a new inspecting technology to investigate and evaluate starch gelatinization through integrating conventional image analyzing and ANNs into one system. Based on the birefringence change using microscopy observation, phase transitions of gelatinization were measured by computer and manual version, respectively, and their performances were systematically compared. In this paper, a new ANN method, starch-SSD, was developed to ensure credibility and robustness of the task by computer reorganization. This study would be useful for a deep understanding of starch gelatinization and potentially provides a valuable way for controlling the modification and synthesis of starch based system at specific conditions.

2. Materials and methods

2.1. Materials

The cornstarch, which contained 74% amylopectin, used in this work is commercially available and was supplied by Penford Food Ingredients Company (Australia). An infra-red heating balance (Model DHS-20, Zhejiang Precision Instrument Co. Ltd, China) was used to measure the moisture content by heating samples to 110 °C for 20 min, and the initial moisture content of the sample was determined to be approximately 13.3%.

2.2. Optical microscopy

A polarization microscope (BHS-2, Olympus Vanox, Japan) equipped with a Micropublisher 3.3 RTV camera and a hot-stage (THMS600, Linkam, UK) thermos system was applied in this work. The magnification was $500 \times (50 \times 10)$. Suspensions of 0.5 wt % starch (with respect to the total amount) were initially prepared in glass vials, and then, one drop of sample was transferred onto a glass cover-slip and sealed using silicon adhesive before being replaced in the hot stage. Fig. 1 shows the typical micrographs of cornstarch observed under both normal and polarized light at specific temperature.

After preparation, the starch suspensions were heated to 100 °C at 2 °C/min. The camera interval timer was set as 30s so that one image was captured per 1 °C temperature increase.

2.3. Modeling and classification of birefringence using ANNs

This section describes the proposed new framework for detection and the associated training methodology. As one of the ANNs method, Single Shot Multi-Box Detector framework (SSD) (Liu et al., 2016) has been applied to detect natural objects such as dog, cat and pedestrian, etc. The SSD approach is based on a feed-forward deep convolutional network that produces a fixed-size collection of bounding boxes and scores for the presence of object class instances in those boxes, followed by a non-maximum suppression step to produce the final detections.

In this study, a new methodology, namely starch-SSD, developed from SSD, is designed to detect and investigate the birefringence change of gelatinization process efficiently. The architecture of the method is shown in Fig. 2. It mainly consists of three parts: data collection, feature extraction and detection, and evaluation of starch gelatinization. It is worth to note that tens of thousands of native and modified starch granules images, which were of various types, regions and even from different processing stage, etc., were collected and kept in the data center of cloud for data storage, management and processing. In short, the data center is a large