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VALIDATION OF A MODIFIED STARCH-IODINE TEST FOR RAPID DETERMINATION OF STARCH CONTENT IN EMULSION LUNCHEON SAUSAGE

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ABSTRACT

Starch is commonly used at a rate of not more than 5% in the formulation of luncheon sausage, which is needed for emulsion stabilization. However, many manufacturers increased their rate of addition as a cost-cutting measure, which is considered a means of adulteration. As a result, this study aimed to develop a reliable, fast, and accurate test for determining starch content in luncheon sausage, as well as to compare the results of this test with those of chemical analysis in order to determine its validity. To accomplish this goal, 2 mm thin slices of emulsion sausages containing 5, 10, 15, 20, and 25% starch were sprayed with a standard iodine solution. The time required for the blue color to fully disappear was calculated, the color intensity was photographed, and a color plate was developed. One thousand emulsion-type luncheon sausage samples belonging to different processing plants were examined for their starch content using both the developed color sheet and the chemical method. The produced sheet's results for > 25% and <5% starch were identical to those of the chemical analysis. However, findings for samples containing 5 to 25% starch showed a slightly lower accuracy (91-96%), but thankfully, the fading time provides a valuable method for determining the starch content. Therefore, the iodine test and the developed calibrated color sheet appeared to be an accurate, rapid, and reliable test for the quantification of starch in luncheon sausage.

Keywords: Emulsion sausage, luncheon, starch, field test, iodine.

INTRODUCTION

Traditional Egyptian luncheon is a large diameter, non-fermented, cooked semi-dry emulsion-type sausage known for its ability to slice into extremely thin slices and high binding properties. Under fair conditions, the intact loaves can be kept sound for 6-8 weeks at room temperature (25 ˚C). The key issue with this product in the past was microbial spoilage as a result of poor hygiene during processing. However, rising demand for processed meat products, a meat supply shortage, and a rise in the meat price, as well as a slight fluctuation in the price of meat products, forced many producers to substitute high-quality beef with various cheaper non-meat ingredients.

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The use of fillers e.g. starch was a common solution to realize this goal.

Starch is a multi-purpose non-meat component that is added during the processing of some meat products. In general, starch creates smooth and tender gels and therefore, is used to increase consistency, improve the overall quality, and prolong the shelf life of finished meat products at a lower cost (Joly and Anderstein, 2009). In meat batter, starch is gelatinized if heated in a humid environment, therefore, boosts the batter viscosity, immobilizes the fat radicle, and binds the water released due to the thermal contraction of proteins (Bañon *et al.,* 2008; Brewer, 2012; Ackar *et al.,* 2015). Furthermore, starches enhance cooking yield, increase binding characteristics, emulsion stabilization, and ultimately maintain juiciness and tenderness (Peris-Tortajada, 2018; Egharevba, 2019). The addition of a high starch content (<5%) is, however, considered a sign of adulteration. It also deteriorates the emulsion sausage slicing capacity and resulting in a dry product (Sison and Almira, 1975). It also results in a starchy appearance, particularly if the loaves are stored stuck with insufficient air in between (personal observation).

Several methods were used to screen or determine starch content in meat products. The screening methods include microscopic staining analysis e.g. histochemical Lugol Calleja staining (Pospiech *et al.,* 2014) and Periodic Acid-Schiff (PAS) stain (Mohamed *et al.,* 2015), and scanning electron microscopy (Eliášová *et al.,* 2012). However, the quantitative tests used for the estimation of starch content in meat products include both the polarimetric titration and the enzymatic methods (ISO 13965, 1998). All these methods are either time-consuming or present technical difficulties due to the formation of abnormal colorations or overestimation of results due to the formation of reducing compounds. Therefore, the present study was designed to develop a reliable on-spot starch-iodine test for rapid quantification of starch in emulsion-type sausage and to correlate the results of this test with those of chemical analysis to estimate its validation as a rapid test for estimation of starch in meat products.

MATERIALS AND METHODS

1. Raw materials

Frozen Brazilian beef neck meat blocks were purchased from a local supplier and kept frozen at -18 °C till use. Mesenteric beef fat was collected immediately after slaughter and carcass preparation from El-Bassaten abattoir, Cairo, Egypt. The fat was washed and stored frozen at -18 ºC. Corn starch (70% amylose content and 1.2% native lipid content) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid, sodium nitrite, and sodium tripolyphosphate were obtained from Loba Chemie (Mumbai, India). Spice oil extracts were purchased from Nubassa GewürzwerkGmpH (Viernheim, Germany). However, sodium chloride was obtained from local markets.

2. Preparation of emulsion luncheon sausage

Control luncheon sausage batter was prepared following the Good Manufacturing Practices Guidelines with the rate of 60% beef, 12.5% added beef fat, 1.5% sodium chloride, 3000 ppm tri-polyphosphates, 500 ppm ascorbic acid, 100 ppm sodium nitrite, 5% corn starch, and 20% crushed ice in addition to quantum sufficient of the spice mix. Both frozen beef blocks and beef fat were first flacked and then minced at a 5 mm Ø mincing plate using a Seydelmann meat grinder (WD 114). Minced beef was chopped at 4000 × g using a K 40 Seydelmann bowel chopper equipped with digital display DGA 33 for temperature and running time guarantees with sodium chloride, sodium tripolyphosphate, sodium nitrite, ascorbic acid, the spices mix. Before crushed ice was added. Minced fat was

added just before 0 ºC, and finely the starch was added at 2ºC with chopping to 8 ºC final batter temperature. At the same time, emulsion sausage batter with 10, 15, 20, and 25% starches were also prepared using the same technique. All the prepared sausage batters were filled into 3 kg loaves using 85-mm diameter impermeable polyamide casing (Viskase, Walsroder casing, Germany,) kept in stainless-steel molds and cooked using a humid cooking program started at 75 ºC for one hr., 85ºC for one hr. and finally 90 ºC till complete cooking. After cooking, the luncheon sausage was showered with cooled water for 30 minutes and stored at room temperature until investigation. Each trial was produced in three patches at different times.

3. Preparation of the test solution and development of the standard color sheet

Different iodine solutions were prepared by dissolving 4g potassium iodide in either 30 ml warm water or ethanol with gentle stirring until completely dissolved, then 2 g iodine crystals were added, and the mixture was shaken until the crystals were thoroughly dissolved, then the mixture was diluted to 1Lwith water or ethanol, mixed well and kept in dark bottles, where different concentrations (0.5-2.5%) were prepared and spared over 2 mm thin slices from the experimentally prepared luncheon sausage. The color intensity, as well as the time necessary for the complete disappearance of the color, were observed. The following color sheet (Plate 1) showed the color intensities after the spraying of 0.5% iodine solution in water by 1, 5, and 15 min for all the trials.

4. Chemical estimation of starch content

Twenty grams of minced luncheon samples were digested in 100 ml alcoholic KOH (8% w/v- 70-80 °C) for 45 min. before being cooled, centrifuged, and filtrated. The aliquot was mixed for 2.5 h with 100 ml 1N Hcl and neutralized with NaOH to pH = 6.3. After centrifugation, the precipitate was washed with ethyl alcohol, centrifuged, and filtered. Three ml each of potassium iron cyanide and zinc acetate solutions were added, and the mixture was allowed to rest for 30 min. before being finished with water to 25 mL. A 100 mg test portion was incubated at 100 °C for 15 min. with 10 ml Na acetate buffer and 0.1m thermostable amylase, then cooled before 0.1 ml amyloglucosidases was added and incubated again at 50 °C for 30 min. before centrifugation. One ml test solution was diluted with 4 ml buffer, and 0.1 ml aliquot was mixed with 3 ml glucose oxidase/peroxidase, and the absorbance was estimated at 510 nm against a blank (AOAC, 2007).

5. Validation of the starch iodine test

One thousand market emulsion-type luncheon sausage samples belonging to ten different processing plants were examined for their starch content using both the 0.5% iodine solution and the developed color sheet (Fig. 1), as well as the chemical method for quantitative estimation of starch. The results of both tests were correlated to evaluate the validity of the developed sheet.

Statistical analysis

The starch content of market samples was determined in triplicate for each sample and the mean value was recorded as Mean ± SE using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

According to the standard plate created by spraying the experimentally produced luncheon sausage with a 0.5% iodine solution (Fig. 1) the strength of the formed color increased from light blue at 5% to blue at 10% and dark blue at higher starch concentrations. The plate showed that the differentiation in the intensity of the developed color at higher starch concentrations was difficult. The results indicated that the time required for the complete disappearance of the formed color appears to be a good estimate of the correct

starch material. In the case of the 5% starch formulations, the color began to fade after 5 min. and was disappeared after 15 min. During the first 5 min, however, the rate of color fading was slower with 10% sausage. At higher concentrations, 5 min was insufficient to judge color fading, but after 15 min, it was very easy to distinguish between the various concentrations.

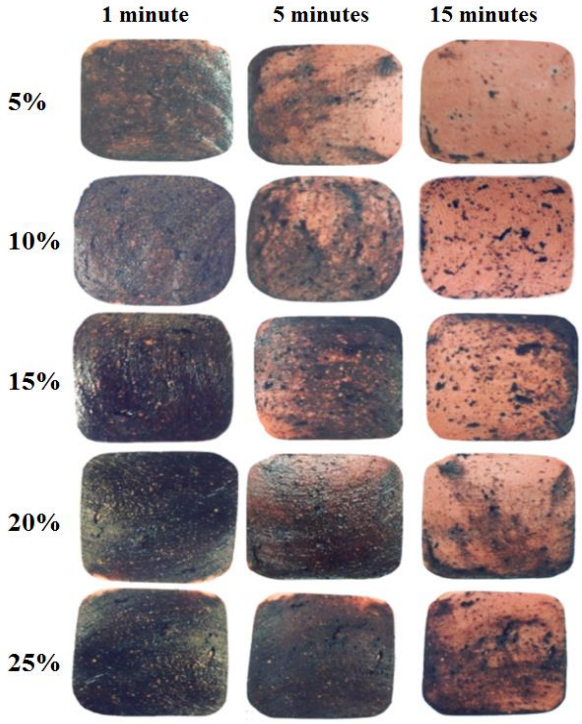

Fig. (1): Standard plate of starch-iodine test of experimentally prepared emulsion luncheon sausage.

Depending on both the intensity and the fading time of the developed blue color, the market samples were categorized into 6 groups (Table 1). The 1st group showed a more intense dark blue color than the maximum color developed in the standard sheet, and the color stayed more than 20 min before it began to diminish indicating that the starch content was more than 25%. Unfortunately, the plate did not contain a standard photo for emulsion with < 25% starch because it was not expected to find such a very high percentage in market samples, a matter which doesn't reduce the validity of the test because under any circumstances starch content higher than 5% is not allowed. The 2nd group had a dark blue color that disappeared after 20 min which was supposed that it had about 25% starch. The time necessary for complete fading of the color effectively divided the samples with 10-20% starch into 3 groups. The time was 15, 10, and 5 min in samples with 20, 15, and 10% starch respectively. Finally, the last group had a light blue color fade within 3.5 min. indicating that the starch content was below 5%.

Examination of emulsion sausage for starch content using the starch-iodine test and the standard plate revealed that the results for samples containing more than 25 and those containing not more than 5% starch were identical to those obtained by chemical analysis (Table 1). This high percentage of accuracy may be due to the role of both the developed color and time of fading in estimating the starch percentage. However, results for samples containing starch percentages between 10 and 25 showed a lower accuracy probably due to the difficulties in determining both the intensity of the color and the time for complete fading of the developed color.

Table 1: Correlation between chemical analysis and starch-iodine test.

Starch/iodine test				Chemical analysis		Accuracy%
No.	*Color*	*Fading time min.*	*Starch%*	*No.**	*Starch% Mean±SE*	
230	Dark blue	30	<25	230	29.99±3.21	100
258	Dark blue	25	<25	258	26.66±2.93	100
151	Dark blue	20	25	145	25.35±3.55	95
145	Blue	15	20	140	21.95±1.88	96.55
74	Blue	10	15	70	17.07±2.03	94.59
61	Blue	5	10	59	15.89±1.25	96.72
72	Blue	2.5	10	66	8.66±2.42	91.66
9	Blue	0.5	5	9	4.87±0.59	100

* The number of samples gives similar results in both tests

DISCUSSION

Starch can be added to emulsion-type meat products at levels up to 5% to perform several technological effects (Luallen, 2018; Wu *et al.,* 2019; Perira *et al.,* 2020). However, some processors added higher levels to reduce the cost of the formula, which deteriorates the quality of the product (Álvarez *et al.,* 2007) and is also considered a mean of adulteration (Souza *et al.,* 1990), a matter which necessitates the existence of an accurate and fast method for the determination of starch in meat products. The two polymers of starch are amylose and amylopectin. The amylose molecules are responsible for the starch's iodine affinity, which results in the development of a complex colored compound (Carciofi *et al.,* 2012), a phenomenon that could help detect the presence of starch in meat products.

Despite the technological functions of starch, it also has some health benefits due to its poor digestibility (Nugent, 2005; Priebe, *et al.,* 2018). However, starches may constitute many potential health hazards (Hendrich, 2018) probably due to the gelatinization during thermal processing in modern processing techniques as in the Egyptian luncheon. Therefore, starch content must not exceed the concentration necessary for performing the emulsion stabilizing effect, which makes the development of a reliable, rapid, and effective method for its estimation a crucial issue. The developed color plate may provide a useful tool for the rapid estimation of starch.

CONCLUSION

The pilot experiments explored that using 0.5% iodine solution in water was the best for differentiation between the different starch concentrations and provided a time-space enough to read the result. It can be safely concluded that the starch-iodine test and the developed standard plate seem to be sensitive and accurate tests for the detection of the adulteration of luncheon sausage with starch beyond the recommended level. The accuracy percentage reached 100% for both low and very high starch levels. However, the rate of accuracy is somewhat lower with the moderated starch contents due to difficulties in evaluating the color intensity and time of fading of the color.

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التحقق من دقة اختبار النشا واليود المعدل للتحديد السريع لمحتوى النشا في مستحلبات اللحوم

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يستخدم النشا بنسبة لا تتعدى 5% من المكونات الكلية للمنتج كمادة مثبته للمستحلب المتكون في اللانشون. ونظراً لرخص سعر النشا مقارنة بالمكونات الأخرى ولما لها من تأثيرات أكثرها إيجابية على جودة اللانشون إتجه العديد من منتجي اللانشون لإضافة النشا بمعدلات عالية جداً بغرض تقليل تكلفة الإنتاج وزيادة هامش الربح مما جعل الأمر يخرج عن كونها مادة مضافة, لها وظيفة تقنية محددة إلى كونها مادة مالئة, وهو الأمر الذي يعد أسلوباً من أساليب الغش والتدليس. ونظراً لكون الطرق الكيميائية والهستولوجية المتعارف عليها لتحديد نسبة النشا في مصنعات اللحوم مكلفة وتحتاج وقت طويل لذلك هدفت هذه الدراسة إلى تطوير اختبار موثوق وسريع ودقيق لتحديد محتوى النشا في اللانشون وهو من أكثر منتجات اللحوم التى تضاف إليه النشا بنسب عالية لخفض التكاليف كما تهدف الدراسة أيضاً لمقارنة نتائج هذا الاختبار بنتائج التحليل الكيميائي لتحديد دقة وموثوقية تلك الطريقة الجديدة. ولتحقيق الغرض من الدراسة تم رش شرائح رقيقة من المنتج النهائي من اللانشون (سمك 2 مم) والذي تم إنتاجة معمليا باستخدام نسب مختلفة من النشا (5، 10، 15، 20 و 25٪) بمحلول قياسى من اليود تم إجراء إختبارات أولية عليه لتحديد التركيز المناسب للإستخدام. وبعد معالجة شرائح اللانشون بمحلول اليود يتم تحديد تركيز اللون الأزرق المتكون نتيجة تفاعل اليود مع النشا وكذلك حساب الوقت اللازم لاختفاء اللون الأزرق المتكون تمامًا، وتم تصوير اللون الأزرق المتكون في شرائح اللانشون المنتج بمستويات مختلفة من النشا في أوقات محددة بعد المعالجة وإعداد شريحة قياسية لذلك. ثم بعد ذلك تم فحص عدد ألف عينة من اللانشون البقري المعروض بالأسواق لتحديد مستوى النشا بها باستخدام كل من الصورة القياسية التى تم إعدادها بعد رشها بمحلول اليود القياسي وبالطريقة الكيميائية القياسية. حيث أظهرت النتائج تطابق تام للنتائج بين كل من الطريقتين في المنتجات التى تحتوي على نسبة من النشا أقل من 5% أو أكثر من 25% من حيث درجة اللون المتكون والزمن اللازم لتلاشي اللون، بينما أظهرت نتائج العينات التي تحتوي على 5 إلى 25٪ نشا دقة أقل قليلاً (91-96٪) ، ولكن لحسن الحظ، يقدم وقت اللازم لتلاشي اللون مساهمة هامة لتحديد محتوى النشا. لذلك خلصت الدراسة إلى أن استخدام محلول اليود القياسي مع الصورة القياسية التى تم الحصول عليها بفحص منتج اللانشون المحتوي على نسب معروفة من النشا اختبار دقيق وسريع وموثوق لقياس كمية النشا في اللانشون.

الكلمات داله: لانشون، نشا ، اختبار ميداني ، يود