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Proportion of common wheat kernels in commercial samples of durum wheat by chemical methods

Introduction

Durum wheat (Triticum durum Desf.) is used for production of semolina which is the required raw material for the elaboration of good quality pasta products. Common wheat (T. aestivum L.), generally used in breadmaking, yields milling products (flour or farina) that are not suitable for that purpose. Shortage and localization of durum wheat production, as well as its higher cost, have compelled the utilization of common wheat for the production of macaroni. When different blends of hard or soft wheat (farina or flour) with durum wheat are used, the quality of macaroni is reduced. For this reason, the estimation of common wheat in pasta products is an important problem from the point of view of quality and market control.

Chemical methods for detection and estimation of common wheat in pasta products have been developed in this laboratory during the past six years. The related problem of estimating the proportion of common wheat kernels in commercial samples of durum wheat has been traditionally solved either by morphological examination or by cytogenetical methods. The former are too unreliable and the latter too cumbersome.

In this paper, we have reviewed the above mentioned chemical methods and investigated their application to the analysis of single kernels and the estimation of the proportion of common wheat kernels in commercial samples of durum wheat in order to avoid the cytogenetical methods or to confirm the morphological examination.

Estimation of common wheat in pasta products

In this laboratory, four methods have been proposed for detection and estimation of common wheat in macaroni (1, 2, 3, 4, 5). These are based on the analysis of certain biochemical differences between the endosperms of common wheat and durum wheat. The development of these methods has consisted of the following steps: (a) Selection of potential biochemical interspecific differences between the two wheat species, based on a comparative biochemical study of composite samples; (b) Design of a simple and reproducible procedure for the analysis of the selected differences; (c) Investigation of possible quantitative variation of the biochemical characteristic due to the milling or the pasta processing; (d) Survey of a high number of varieties from both species in order to establish the tentative interspecific limits of variation of the biochemical difference, so that the maximum and minimum possible common wheat content in an unknown mixture can be calculated as a function of the observed value of the biochemical characteristic. In our opinion, all four requisites have to be met if a proposed method is to be of any practical value.

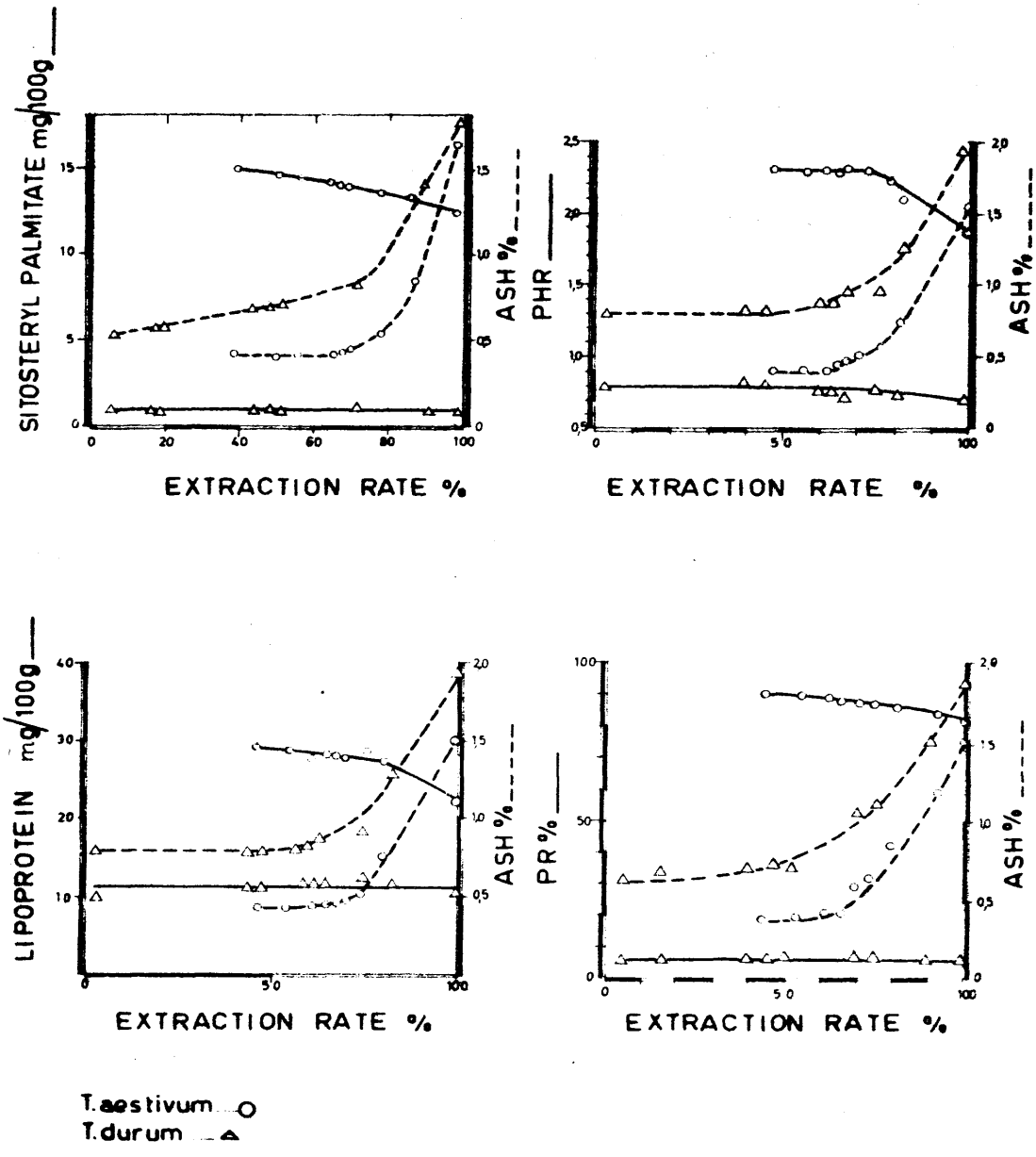
Furthermore, we have investigated the genetic basis of the interspecific biochemical differences (6, 7, 8, 9, 10). These data are relevant in connection with the possible obsolescence of the proposed tests as a result of the release of new varieties obtained by the more recent breeding methods.

The four selected differences are: (1) sitosteryl palmitate content; (2) an electrophoretic component from water extracted proteins (PHR); (3) total light petroleum extracted lipoprotein; and (4) an electrophoretic component from chloroform:methanol (2:1) soluble protein (PR).

The variations of the four biochemical differences as a function of the extraction rate are plotted in Figure 1. Normal variations of extraction rate do not affect any of them. Only PHR and the lipoprotein show a significant difference between the value corresponding to the 70 % extraction rate and whole wheat.

In Figure 2, interspecific limits of variation are represented. The maximum and minimum possible content of common wheat of an unknown mixture are plotted as a function of the observed values of the biochemical differences. Also plotted are the magnitude of the intervals of uncertainty. It can be concluded from these data that the PR index (chloroform:methanol protein) is the most satisfactory if a single method is to be used.

Figure 1.



Analysis of single kernels

We have adapted the chemical methods for the estimation of common wheat semolina or in pasta products to the analysis of single kernels. Procedures are summarized in the following paragraphs:

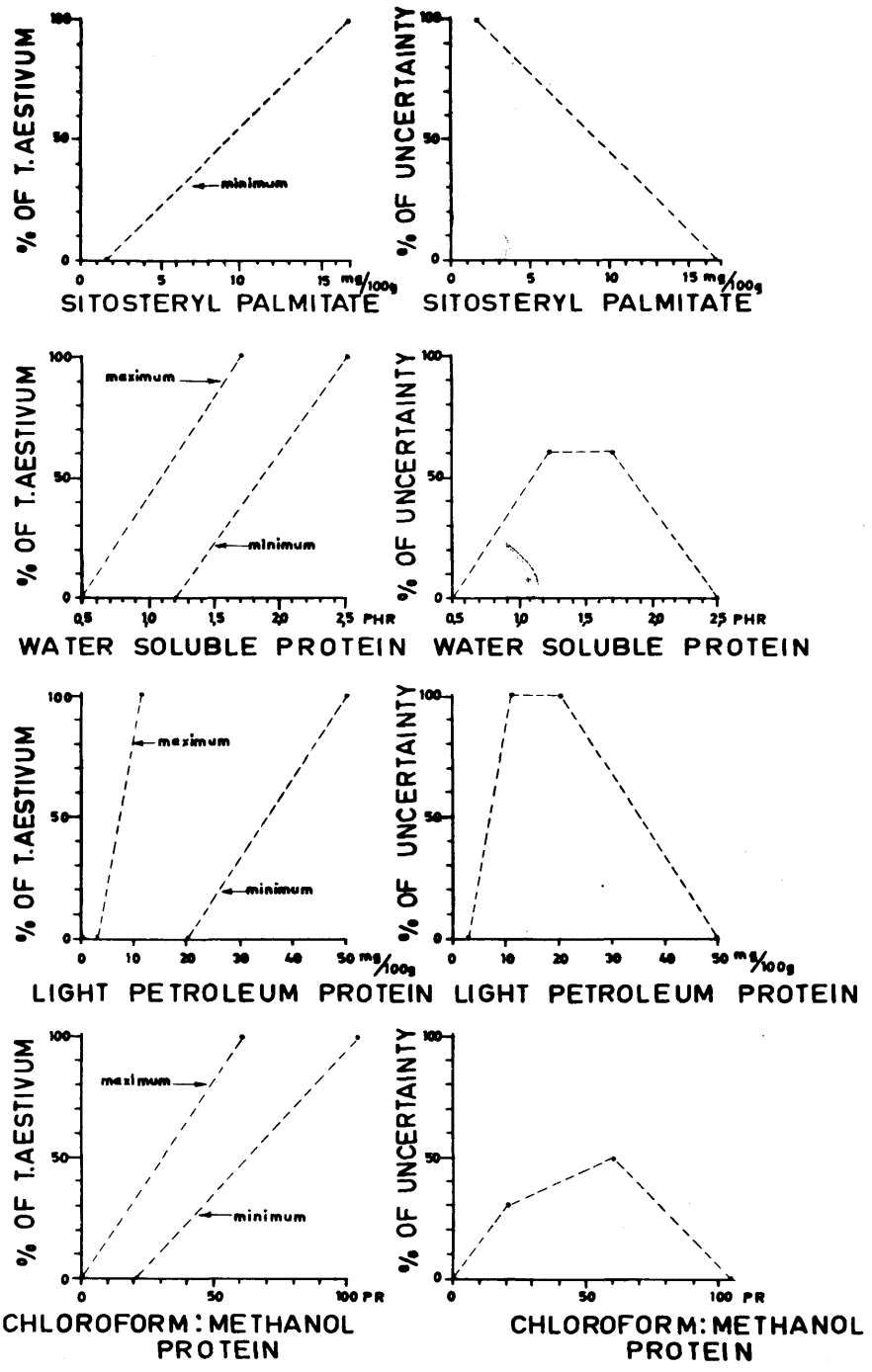
1. Sitosteryl palmitate

Single kernels are placed between two well polished metal plates, crushed by one hammer blow, and transferred to a small test tube. The crushed kernels are extracted for 2 hours with .25 ml of petroleum ether (b.p. 35...60° C). The supernatant is spotted with the aid of a capillary tube into a silicagel G thin layer (0.2 mm thick). Development is carried out with carbon tetrachloride (11) and visualization with iodine vapor.

2. Water soluble proteins

The kernels are crushed as above and macerated with water for 21 hours at 4° C. A piece of paper (Whatman No. 3, 2 x 8 mm) is soaked in the extract and inserted in the gel slot. Starch gel electrophoresis is performed according to WOYCHIK et al. (12).

Figure 2.



3. Light petroleum extracted lipoproteins

The ground kernels are macerated for two hours with .25 ml of petroleum ether (b.p. 35...60° C). The supernant is transferred with the aid of a capillary tube to a piece of filter paper (Whatman No. 3, 2 x 8 mm) and evaporated in the process. Lipid is dissociated from protein by treating the paper with 1 N HCl in ethanol:petroleum ether (3:1) with the aid of a capillary and then is extracted by immersion in petroleum ether for 1 hour. The dried paper is wet with buffer, and inserted in the electrophoresis gel.

4. Chloroform:methanol (2:1, v/v) protein

The crushed kernels are defatted with .25 ml of petroleum ether (b.p. 35...60° C) and extracted with .15 ml of chloroform:methanol (2:1, v/v). The extract is transferred with the aid of a capillary to a piece of filter paper (Whatman No. 3, 2 x 8 mm) and evaporated in the process. The absorbed protein is subjected to starch-gel electrophoresis as above.

Choice of method

After application of the methods just outlined, the following conclusions can be drawn: Sitosteryl palmitate determination is the easiest to perform, but it has been shown that this character is controlled by a single gene (6) and is absent from some T. aestivum varieties. The electrophoretic patterns of water soluble proteins from the whole kernel, although showing differences for both wheat species, these are not as clearcut as when only the endosperm is analyzed.

The electrophoresis of lipoprotein shows the main component of this fraction, the purothionins. These are detected in Triticum aestivum kernels, but the method is not sensitive enough for detection in durum kernels. Furthermore, common wheat varieties with low purothionin content and small kernel size might not be detected. Electrophoretic patterns of chloroform:methanol proteins from whole kernels and from the endosperm are identical. Common and durum wheat kernels are readily differenciated by this method and, consequently, is the method of our choice. It should be pointed out that it can be applied sequentially with the sitosteryl palmitate or the purothionin methods because these components can be analyzed in the petroleum ether extract and the chloroform:methanol protein in the defatted residue.

Proportion of common wheat kernels in commercial samples of durum wheat

Common wheat present in durum wheat samples can be detected by milling of the sample and application of the chemical methods used for flour or pasta products. However, the availability of methods for the analysis of single kernels means that a greater precision can be gained by this new approach. In Table 1, the 95 percent confidence intervals for different sample sizes are summarized. The maximum intervals for each sample size corresponds to the .50 proportion. For sample size 20, the maximum 95 percent confidence interval is of the same order as the maximum interval of uncertainty in the most favorable case of Figure 2. A great precision is achieved with sample size 1000. Although it might not be practical to perform the chemical analysis of 1000 kernels, this difficulty can be bypassed by the previous stratification of the sample in morphologically homogeneous classes and identification of classes by chemical analysis.

Table 1. 95 percent confidence intervals for different sample sizes

Observed proportion	Size of sample							
	20		50		100		1000	
.10	.01	.31	.03	.22	.05	.18	.08	.12
.50	.27	.73	.36	.64	.40	.60	.47	.53

Summary

The problem of estimating the proportion of common wheat kernels in commercial samples of durum wheat has been traditionally solved either by morphological examination or by cytogenetical methods. The former are too unreliable and the latter too cumbersome.

Chemical methods for detection and estimation of common wheat in pasta products have been developed in this laboratory during the past six years.

In this paper, we have investigated the application of the above chemical methods to a single wheat kernel in order to avoid the cytogenetical method or to confirm the morphological examination.

Analysis of sitosteryl palmitate and/or chloroform:methanol proteins are easily performed on a single kernel, but the latter is more suitable because no exceptions have been found up to now for the biochemical interspecific difference used.

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