Microbiological and mycotoxicological quality parameters of naked and covered oats with regard to the production of bran and flakes

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Mikrobiologische und mykotoxikologische Qualitätsparameter von Nackt- und Spelzhafer in Hinsicht auf die Produktion von Kleie und Flocken

1. Introduction

Due to their favourable nutrient composition and specific fiber components (high ß-glucan content), oat products constitute a valuable foodstuff. Moreover, recent research work has shown that oats may safely be included in a gluten-free diet for celiac disease patients (HOFFENBERG et al., 2000; PICARELLI et al., 2001). For these reasons, a growing portion of a health-conscious population is showing a keen interest in oat products such as oat flakes and bran.

Oat flakes and bran are obtained either from naked oats or covered oats. As a basic product, naked oats has some morphological and technological advantages over covered oats. The kernels of naked oats are somewhat larger, storage and transport are less costly, no hulling is required, and no offal is produced which might create a disposal problem (BURROWS, 1986). Studies carried out in the Czech Republic over several years have shown that under the conditions prevailing there, more flakes can be produced from naked oats despite the relatively lower quan-

Zusammenfassung

Ziel der Untersuchung war es abzuklären, ob Nackthafer oder Spelzhafer der bessere Ausgangsrohstoff für die Haferkleie- bzw. Haferflockenproduktion in mikrobiologischer und mykotoxikologischer Hinsicht ist. Dazu wurde die mikrobielle Qualität der Ernteproben von je drei Nackthafer- und Spelzhafersorten an vier Standorten in zwei Vegetationsperioden geprüft. Als Parameter der mikrobiellen Qualität wurden die Bakterien- und Pilzkeimzahlen, Ergosterin als chemischer Gradmesser der Verpilzung, sowie die Kontamination mit den wichtigsten Fusarientoxinen herangezogen. Bei den Spelzhafersorten erfolgte die Qualitätsprüfung vor und nach dem Entspelzen.
Die Ergebnisse zeigen, dass durch das Entspelzen die Belastung der Haferkörner mit Mikroorganismen und Mykotoxinen drastisch reduziert wird, sodass die entspelzten Kerne von Spelzhafer eine bessere mikrobielle Qualität, vor allem hinsichtlich der Kontamination mit Fusarientoxinen, aufweisen als die Kerne von Nackthafer.

Schlagworte: Hafer, Entspelzung, Fusarientoxine, Bakterien- und Pilzkeimzahlen, Ergosterin.

Summary

The object of the study was to clarify whether - from a microbiological and mycotoxicological point of view – naked oats or covered oats was the better starting material for the production of oat bran or flakes. To this end, the microbial quality of samples of three cultivars of naked, and three cultivars of covered oats, collected at a total of four locations was tested over two vegetation periods. The parameters used to measure the microbial quality were: the bacterial and fungal counts, ergosterol as a chemical indicator of fungal attack, as well as contamination with the principal *Fusarium* toxins. In the cultivars of covered oats, the quality tests were carried out before and after hulling.
The results show that hulling will bring about a drastic reduction in the contamination of oat kernels with microorganisms and mycotoxins. Consequently, the hulled groats of covered oats show a higher microbial quality than those of naked oats, especially with regard to their contamination with *Fusarium* toxins.

Key words: oats, hulling, *Fusarium* toxins, bacterial and fungal counts, ergosterol.

tities harvested when compared to covered oats (MOUDRY, 1998).

On the other hand, given the exposed nature of the karyopsis, naked oats is more sensitive to damage caused by threshing, storage and transport as well as to chemical degradation. Another negative technological aspect in oat flake production is the fact that naked oats will always contain certain quantities of kernels with the hulls still attached.

An important factor that has so far gone unheeded in the difficult choice between naked and covered oats is the microbial quality of oat products, i.e. their degree of mycotoxin contamination. The morphology of the grain on the standing crop makes covered oats particularly vulnerable to bacteria and fungi, because the oat hulls (palea and lemma) firmly enclose the oat kernel (groat) without, however, actually adhering to it. It is from this sheltered space that especially the black (sooty) moulds attack the inside of the hulls where the cells are weaker and have thinner walls (SCHMIDT, 1981). However, the first target of the fungal attack will be the faded anthers, which in a high percentage of oat grains at the time of harvest are still enclosed by the hulls (LEW, 1995).

Beside this largely epiphytic or saprophytic fungal growth, oats is often heavily infested by pathogenic and toxinogenic fusaria such as *F. graminearum*. This fungus, which also acts as the main producer of the toxins deoxynivalenol (vomitoxin) and zearalenone, will infect the oats already during the flowering period, with moist and sultry weather promoting the infection. Moreover, oat grains are frequently contaminated with nivalenol, which on oats is mostly formed by *Fusarium poae* (PETTERSSON, 1991; LEW et al., 1997).

In the case of covered oats, the kernels are not separated from the hulls by threshing so that the entire fungal and mycotoxin contamination – which can be extremely massive – will remain in the threshed crop. By contrast, the spikelets of naked oats, whose thinner and looser hulls ensure faster drying after rainfalls, do not offer such ideal conditions for the growth of microorganisms. Besides, threshing has the effect of shaking most of the kernels out of the hulls with their high susceptibility to fungal attack.

On the other hand, the groats of covered oats are hulled prior to the production of oat flakes and bran, which will eliminate the mouldy hulls and, as part of the cleaning process taking place in the mill, also the heavily infected shrivelled kernels which are particularly contaminated with mycotoxins.

The primary goal of the project, therefore, was to establish whether from the point of view of microbial quality, hulled covered oats or naked oats was more suitable as a raw material for oat bran and oat flakes.

The parameters determining microbial quality were bacterial and fungal counts, ergosterol as a chemical measure of fungal attack, as well as contamination with the *Fusarium* toxins deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), T-2 toxin (T-2), HT-2 toxin (HT-2) and zearalenone (ZON).

2. Material and methods

In the project at hand, the microbial quality (i.e. the contamination with microorganisms and *Fusarium* toxins) of three cultivars of naked oats and three cultivars of covered oats was tested during two vegetation periods at two locations in the Czech Republic and two in Austria (Table 1).

Each cultivar was grown in 4 replications in plots of 10–12 m^2 each, with the usual cultivation methods typical of the various sites.

Cultivars tested: Auron, Expander, Edmund (covered oats)
Abel, Izak, Salomon (naked oats)

The microbiological and mycotoxinological analyses were performed in grain samples of ca. 1 kg won from each plot. The samples of covered oats were subjected to the analyses before and after hulling, for which a pneumatic dehusker (Rietberg, type HSD) was used. The inevitably occurring husked kernels in naked oat samples were removed by hand prior to analysis.

Table 1: Description of the test fields
Tabelle 1: Beschreibung der Versuchsfelder

Location	Soil type	Altitude	Mean annual temperature	Mean annual precipitation
C. Budejovice (CZ)	Luvisol	380 m	7.8 °C	620 mm
Freistadt (A)	Cambisol	503 m	6.8 °C	694 mm
Humpolec (CZ)	Cambisol	570 m	7.5 °C	650 mm
Lambach (A)	Cambisol	366 m	8.2 °C	957 mm

2.1 Bacterial and fungal counts, identification of *Fusarium* species

Viable counts of bacteria (total aerobic mesophilic counts), mould and yeast counts were determined by means of the surface method according to the IAG (1993) standard methods. Fusaria were isolated on peptone PCNB agar (BURGESS et al., 1988), then transferred onto potato dextrose agar (PDA; Oxoid) and incubated for one week at 28 °C. Subsequently, *Fusarium* colonies were cultivated on SNA (NIRENBERG, 1976) as single spore isolates and identified according to BURGESS et al. (1988) and also according to NIRENBERG and O'DONNELL (1998).

2.2 Trichothecenes of the A-group (HT-2, T-2) and the B-group (DON, 3-ADON, 15-ADON, NIV)

The analysis involved a Mycosep column clean-up with subsequent capillary GC of the HFB and TMS derivatives and EC detection according to LEW et al. (2001a).

Clean up and derivatization
10 g of the sample were mixed with 100 ml of acetonitrile/water (84+16, v+v) and shaken for 120 min before the extract was filtered off. 6 ml of the filtrate were then cleaned up on a Mycosep # 227 column (Romer Labs Inc.). Next, 2 ml of the cleaned extract were evaporated to dryness at 45 °C. The residue was redissolved in 400 µl of acetonitrile and 100 µl each were stored at a temperature below –18 °C prior to halogenation of the A-trichothecenes and silylation of the B-trichothecenes, respectively. Halogenation with heptafluorobutyrylimidazole and silylation with Tri-Sil TBT followed the methodology prescribed by SCOTT et al. (1986).

GC conditions
The GC apparatus was a HP 5890 A equipped with a ^{63}Ni electron-capture detector, an autosampler HP 7673 and a split/splitless injector operating in splitless mode. GC separation was achieved with a 50 m × 0.32 mm i.d. CP-Sil-8CB fused silica capillary column with a 0.1 µm film thickness.

The carrier gas was Helium, the injector and detector temperatures were 250 °C and 300 °C, respectively. The injection volume was 3 µl (in 2,2,4-trimethylpentane).

Temperature programmes:
A-Trichothecenes: Start at 80 °C, hold for 2 min, then increase to 205 °C at 28 °C/min, followed by an increase to 240 °C at 2 °C/min and finally to 290 °C at 25 °C/min, with a final holding time of 6 min.

B-Trichothecenes: Start at 80 °C, hold for 2 min, then increase to 192 °C at 9.5 °C/min, hold for 5 min, followed by an increase to 212 °C at 1 °C/min and finally to 290 °C at 25 °C/min, with a final holding time of 6 min.

2.3 Zearalenone

Zearalenone was determined by means of alkaline clean-up followed by HPLC and fluorescence detection. Clean-up and HPLC determination was performed according to the publication by SCHUHMACHER et al. (1998).

2.4 Ergosterol

Ergosterol was determined by means of HPLC and diode array detection according to SCHWADORF and MÜLLER (1989), with some modifications in the preliminary clean-up step.

10 g of the sample were mixed with 10 g KOH, 50 ml ethanol and 100 ml methanol and subsequently saponified for 30 minutes in a magnetic stirring water bath, at 80 °C. After cooling, the saponified mixture was filtered. 10 ml of the filtrate were mixed with 10 ml of diluted phosphoric acid (25 ml 85 % H_3PO_4 with distilled water ad 1000 ml) and poured onto an Extrelut-column (Merck). After an exposure time of 10 minutes the ergosterol was eluted with 120 ml of hexane. The eluate was then dried over Na_2SO_4, evaporated to dryness at 40 °C and redissolved in 500 µl of toluene for the HPLC.

HPLC conditions:
The equipment consisted of the HP 1050 ChemStation with a diode array detector (HP 1040 M).

The HPLC column was a LiChrospher Si60, 250 x 4 mm, 5 µm. Hexane/iso-propanol (97.5+2.5, v+v) was used as mobile phase under isocratic conditions, with a flow rate of 1.3 ml/min. The detection and the reference wavelength were 282 nm and 580 nm, respectively.

3. Results and discussion

Climate factors, above all the volume of rainfall during the ripening season, have a direct bearing on the microbial contamination of oats. As the cereal ripens and ages, on the one

hand its resistance to bacteria and fungi declines, but on the other hand the gradual drying of the grains as a natural consequence of the ripening process counteracts the growth of microorganisms. This balance, however, will be markedly shifted towards the development and multiplication of microbes by precipitations occurring at the time of full maturity or, worse, of dead ripeness (LENGAUER, 1993).

This becomes vividly evident from a comparison between the bacterial and fungal counts of the oats harvests of 1999 and 2000 (Tables 2 and 3). In the harvest year 2000 the germ counts are clearly higher than in the year before because despite the maturity of the oats being far advanced towards the middle of July 2000, the actual harvest was delayed far into the month of August by a prolonged spell of adverse weather.

All the same, differences in site, weather conditions and cereal cultivar had little to no effect on the composition of the epiphytic and saprophytic fungal flora. In fact, in all oat cultivars and at all locations, and in both years under investigation, the clearly dominating genera of fungi, with but little variation in proportion, were *Cladosporium* and *Acremonium*, followed by *Verticillium*, *Aureobasidium*, *Alternaria* as well as coelomycetes.

As the results of the microbiological analysis have shown, the removal of the hulls causes a dramatic reduction of the microbial contamination of oat grains. Thus, in hulled groats from the harvest 1999 the bacterial counts dropped to 13 %, and the fungal counts to only 7 % of the values for samples of covered oats. These germ counts of the hulled groats were around 37 % and 26 %, respectively, compared to the samples of naked oats (Tables 2 and 3).

However, the ergosterol contents of the naked oat samples were well in line with the results for hulled oat kernels, despite the higher fungal counts (Table 4). Since ergosterol is deemed a more reliable measure of fungal infestation and fungal mass than the fungal count (MÜLLER and LEHN, 1988), one can assume that the analyses of samples from the 1999 harvest did not show any significant differences

Table 2: Total aerobic mesophilic counts (CFU/g in millions), mean values from 4 replications
Tabelle 2: Aerobe mesophile Gesamtkeimzahlen (Mio. KBE/g), Mittelwerte aus 4 Wiederholungen

	Location							
	C. Budejovice		Freistadt		Humpolec		Lambach	
Year of harvest	1999	2000	1999	2000	1999	2000	1999	2000
Auron	51	211	13	387	95	190	83	342
Edmund	39	153	12	212	108	261	106	141
Expander	51	206	23	237	90	172	100	212
Auron, hulled	11	10	3	15	13	33	12	14
Edmund, hulled	7	7	2	10	11	27	12	8
Expander, hulled	10	14	2	14	5	25	12	6
Abel	19	12	5	45	26	20	45	14
Izak	9	28	2	51	32	19	33	11
Salomon	25	15	6	25	20	34	49	17

CFU = colony-forming units

Table 3: Fungal counts (CFU/g in thousands), mean values from 4 replications
Tabelle 3: Pilzkeimzahlen (Tsd. KBE/g), Mittelwerte aus 4 Wiederholungen

	Location							
	C. Budejovice		Freistadt		Humpolec		Lambach	
Year of harvest	1999	2000	1999	2000	1999	2000	1999	2000
Auron	71	786	38	690	153	666	82	458
Edmund	91	662	60	395	190	481	73	349
Expander	66	591	51	484	98	439	81	543
Auron, hulled	7	20	7	11	12	76	6	46
Edmund, hulled	9	18	4	10	5	43	9	28
Expander, hulled	7	18	2	6	6	62	4	18
Abel	27	21	12	55	61	69	20	20
Izak	16	105	12	31	45	44	15	19
Salomon	22	88	7	20	47	40	21	17

CFU = colony-forming units

between the two raw materials for oat products, as regards their epiphytic and saprophytic fungal development. The higher fungal counts in the naked oats samples, when compared to the hulled groats, are probably due to a higher degree of sporulation of the fungi infesting the naked oats, since spores tend to develop preferably on unobstructed surfaces.

An even clearer reduction of germ counts due to hulling was observed for the harvest year 2000. Here, the bacterial counts in the hulled groats fell to 7 %, and the fungal counts to a mere 5 % of the initial values observed for covered oats. The bacterial and fungal counts of the naked oats samples were only 10 % and 8 %, respectively, of the figures for covered oats. This would allow the conclusion that the microbial quality of covered oats suffered particularly severely from the adverse weather conditions of July 2000.

In keeping with the fungal counts, the ergosterol contents of hulled groats, in the year 2000, were slightly below those of the naked oats samples.

Deoxynivalenol (DON) is the most frequent *Fusarium* toxin affecting cereals in Europe (ERIKSEN and ALEXANDER, 1998) while *Fusarium graminearum* is the main DON producer in Central and South Europe. Moreover, this fungus produces the nonsteroidal estrogenic zearalenone. In the *F. graminearum* strains isolated so far from Austrian oats, the biosynthesis of DON passes exclusively via the precursor 3-ADON, whereas *F. graminearum* strains isolated from maize develop DON via the precursor 15-ADON (LEW et al., 2001b; ADLER et al., 2002).

F. graminearum infests the oats already during the flowering period, although it has to be added that usually only single spikelets are actually affected by the fungus. Thanks to the spacing of the spikelets on the panicle secondary infection is a rare phenomenon – quite unlike maize or wheat. If threshing is performed under dry conditions and with careful aspiration and cleaning, the DON contamination of oats should therefore be less serious than in the case of wheat (LEW, 1995).

In fact, in the two years under review, only low DON contents were detected in the samples of oats. The low DON contaminations did not allow any conclusive comparison between naked oats and hulled groats. Only at Lambach – where the menace of a *Fusarium graminearum* infection was felt to be particularly real – were we able to demonstrate in 1999 what qualifies as fairly relevant DON contents (up to 0.53 mg/kg) in covered oats. Here, also the naked oats and the hulled groats did show occasional DON concentrations but never higher than 0.13 mg/kg and 0.12 mg/kg, respectively. The 3-ADON content remained below the analytical quantification limit in all samples, while 15-ADON, as had been expected, was not detected at all. Zearalenone occurred in just one covered oats sample and one naked oats sample, at very low concentrations (0.03 mg/kg).

A more suitable indicator was found in the contaminations of the oat samples with nivalenol and the trichothecenes of the A-group, T-2 toxin and HT-2 toxin. In oats, nivalenol is mainly produced by *F. poae* (PETTERSSON, 1991; LEW et al., 1997); and *F. poae* regularly dominates the *Fusarium* flora on Austrian oats (ADLER, 1993), so that it is small wonder that high nivalenol concentrations have frequently been reported for oats. Like the entire group of trichothecenes, nivalenol acts as an inhibitor of the protein synthesis. As a consequence of a recent toxicological assessment by the Scientific Committee on Food of the EU (SCF, 2000), a temporary tolerable daily intake (t-TDI) of 0.7 µg/kg body weight has been established.

T-2 toxin and HT-2 toxin are judged by the Scientific Committee on Food to be considerably more toxic than

Table 4: Ergosterol contents (mg/kg), mean values from 4 replications
Tabelle 4: Ergosteringehalte (mg/kg), Mittelwerte aus 4 Wiederholungen

	Location							
	C. Budejovice		Freistadt		Humpolec		Lambach	
Year of harvest	1999	2000	1999	2000	1999	2000	1999	2000
Auron	4.8	21.3	4.1	19.3	6.5	11.9	8.5	22.2
Edmund	6.4	19.9	6.9	22.3	7.1	10.6	16.3	19.8
Expander	5.3	19.7	4.6	20.8	7.4	11.6	11.7	26.2
Auron, hulled	0.8	2.1	0.8	1.9	0.7	2.2	2.0	2.2
Edmund, hulled	1.1	2.6	1.1	2.0	0.5	2.0	1.9	1.9
Expander, hulled	0.9	1.5	0.7	1.5	0.7	2.1	1.4	2.3
Abel	1.0	2.7	0.8	3.7	1.1	4.7	1.4	3.2
Izak	0.9	3.3	1.1	4.1	1.3	2.9	1.7	3.6
Salomon	1.0	3.0	1.1	3.7	1.2	4.2	1.8	3.1

nivalenol. Based on their general toxicity, haemato- and immunotoxicity, a temporary TDI value of 0.06 µg/kg body weight has been calculated (SCF, 2001).

Both at C. Budejovice and Lambach, high nivalenol contents (up to 1.86 mg/kg) could be detected in the covered oats harvested in the year 2000 (Table 5). However, the concentration of this toxin in the hulled groats was generally below the analytical quantification limit of 0.1 mg/kg: evidently here the toxin contamination was limited to the hulls. Two-thirds of the naked oats samples collected at C. Budejovice did show moderate nivalenol contents (max. 0.28 mg/kg). By contrast, the nivalenol concentrations observed at Freistadt and Humpolec were both relatively low (up to 0.27 mg/kg) and restricted to the covered oats samples. In the year 1999 nivalenol was occasionally detected in covered oats at Lambach (up to 0.17 mg/kg), C. Budejovice (up to 0.25 mg/kg) and Humpolec (up to 0.39 mg/kg).

What was alarming was the content of T-2 toxin and HT-2 toxin in the year 1999, particularly at Lambach and Freistadt (Tables 6 and 7), given the relatively high toxicity of the two compounds. Similarly to nivalenol, these two toxins were located almost exclusively in the husks. Especially where oats are employed as feed – usually in the unhulled state – one would have to expect an impairment of animal health with such high toxin concentrations.

A major source of T-2 toxin and HT-2 toxin in the samples of oats could be identified in the *Fusarium* species *F. langsethiae* (*F. pulverosum*), only recently described, which from the morphological und chemotaxonomical point of view would have to be classified between the species *F. poae* and *F. sporotrichioides* (TORP and LANGSETH, 1999). While *F. langsethiae* dominated the *Fusarium* flora of the harvest 1999, *F. poae* was the dominant species throughout the harvest year 2000. Besides, both years yielded evidence of *F. avenaceum*, *F. tricinctum, F. graminearum* and *F. sporotrichioides*. On the one hand the naked oats sample, containing up to 0.26 mg T-2 toxin/kg and up to 0.47 mg HT-2 toxin/kg, showed markedly lower toxin contents than the covered oats samples. But on the other hand, as in the case of nivalenol, these figures were clearly above the values observed in hulled groats. Consequently, from the mycotoxicological viewpoint, the groats of covered oats may be considered superior to the groats of naked oats.

Table 5: Nivalenol contents (mg/kg), harvest 2000
Tabelle 5: Nivalenolgehalte (mg/kg), Ernte 2000

Replication	Location							
	C. Budejovice				Lambach			
	1	2	3	4	1	2	3	4
Auron	1.86	0.97	0.84	0.11	0.35	0.18	0.28	1.02
Edmund	0.19	< 0.10	0.17	0.31	< 0.10	< 0.10	0.16	0.12
Expander	0.49	0.24	0.19	0.19	0.14	< 0.10	0.12	0.28
Auron, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Edmund, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Expander, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Abel	< 0.10	< 0.10	0.13	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Izak	0.28	0.16	0.27	0.21	< 0.10	< 0.10	< 0.10	< 0.10
Salomon	0.18	< 0.10	0.21	0.15	< 0.10	< 0.10	< 0.10	< 0.10

Table 6: T-2 toxin and HT-2 toxin contents at the Lambach plots, harvest 1999
Tabelle 6: T-2 Toxin- und HT-2 Toxingehalte am Standort Lambach, Ernte 1999

Replication	T-2 toxin, mg/kg				HT-2 toxin, mg/kg			
	1	2	3	4	1	2	3	4
Auron	0.21	0.29	0.26	0.19	0.95	1.11	1.02	0.95
Edmund	0.16	0.24	< 0.10	0.10	0.99	1.15	0.43	0.68
Expander	0.49	0.38	0.55	0.50	0.84	0.70	0.95	0.93
Auron, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.05	0.05	0.05	< 0.05
Edmund, hulled	< 0.10	< 0.10	< 0.10	< 0.10	0.16	0.11	< 0.05	< 0.05
Expander, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.05	< 0.05	< 0.05	< 0.05
Abel	< 0.10	0.12	0.13	< 0.10	0.33	0.47	0.26	0.23
Izak	< 0.10	< 0.10	0.26	< 0.10	0.43	0.40	0.32	0.20
Salomon	< 0.10	0.14	< 0.10	< 0.10	0.26	0.28	0.06	0.16

Table 7: T-2 toxin and HT-2 toxin contents at the Freistadt plots, harvest 1999
Tabelle 7: T-2 Toxin- und HT-2 Toxingehalte am Standort Freistadt, Ernte 1999

Replication	T-2 toxin, mg/kg				HT-2 toxin, mg/kg			
	1	2	3	4	1	2	3	4
Auron	< 0.10	0.14	0.12	0.20	0.21	0.71	0.30	0.64
Edmund	< 0.10	< 0.10	< 0.10	< 0.10	0.15	0.25	0.22	0.23
Expander	< 0.10	0.32	0.15	< 0.10	0.18	0.64	0.24	0.26
Auron, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.05	0.05	< 0.05	< 0.05
Edmund, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.05	< 0.05	< 0.05	< 0.05
Expander, hulled	< 0.10	< 0.10	< 0.10	< 0.10	< 0.05	< 0.05	< 0.05	< 0.05
Abel	< 0.10	< 0.10	< 0.10	0.13	< 0.05	0.08	0.05	0.22
Izak	< 0.10	< 0.10	< 0.10	< 0.10	0.08	0.17	0.05	0.15
Salomon	< 0.10	< 0.10	< 0.10	< 0.10	< 0.05	0.11	< 0.05	< 0.05

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